UNIVERSITÀ DI SIENA 1240

UNIVERSITA' DI SIENA

DIPARTIMENTO DI MEDICINA MOLECOLARE E DELLO SVILUPPO

DOTTORATO DI RICERCA IN MEDICINA MOLECOLARE

CICLO XXXIII

COORDINATORE Prof. Vincenzo Sorrentino

TITOLO DELLA TESI:

Endometriosis and stress: impact on women's health

SETTORE SCIENTIFICO-DISCIPLINARE: MED/40 – Ginecologia e Ostetricia

DOTTORANDO

Dott.ssa SILVIA VANNUCCINI

TUTOR

Prof. VINCENZO DE LEO

ANNO ACCADEMICO: 2019/2020

SILVIA VANNUCCINI

Firmato il 09/05/2021

Abstract

Endometriosis is a chronic gynecological disorder, defined by the presence of endometrial tissue outside the uterine cavity, which undergoes the same cyclic changes of eutopic endometrium. The prevalence of the disease can reach about 10% of reproductive-age women, who present with chronic pelvic pain and/or infertility, leading to negative physical and psychological impact in patient's lives. The pathogenesis involves several hormonal, inflammatory, immune, genetic, and epigenetic mechanisms, which may play a role in the establishment and progression of endometriotic lesions and their clinical manifestations.

In the last years, a possible link between high levels of chronic stress and endometriosis has been hypothesized. Clinical evidence shows that patients with endometriosis report higher levels of perceived stress, anxiety, and depression than healthy women. Stress levels are positively correlated to the severity of the disease and pain intensity. Women with endometriosis, in fact, report impaired quality of life and they are more likely to be affected also by systemic and gynecological comorbidities, contributing to stress in a vicious circle. A high prevalence of immune, inflammatory, allergic and mental health diseases has been found and a strict link with adenomyosis has been shown. Moreover, experimental studies have shown that previous exposure to repeated and excessive stress accelerates the development and increases the severity of endometriosis in animal models and early life stressor contribute to the development of the disease in adult life. However, it is still debatable whether endometriosis is a cause and/or a consequence of stress and which mechanisms explain this relationship.

An adequate management of endometriosis contribute to improve quality of life, reducing stress levels. It has been shown that among women surgically treated, those with worse quality of life had first surgery at a younger age, more symptoms recurrence and more reoperations. Thus, first endometriosis surgery at a later age is an independent predictor of better health condition. Furthermore, patients experiencing repetitive surgery for endometriosis recurrences have lower physical and mental scores than patients receiving a single surgical treatment. Women undergoing hormonal treatment both before and after surgery have a lower rate of recurrence and an improvement of clinical presentation, in terms of pain symptoms. Thus, hormonal treatments represent a valid cornerstone of endometriosis management and may be useful as a first-line therapeutic approach, in order to postpone surgery, and after surgery itself to reduce the risk of recurrence. The gold standard for modern endometriosis management is the individualized approach, aiming to reduce perceived stress, stress-related comorbidities and to improve quality of life.

Table of content

1. Introduction

Endometriosis is a chronic gynecological disorder, defined by the presence of endometrial tissue outside the uterine cavity, which undergoes the same cyclic changes of eutopic endometrium. The prevalence of the disease can reach about 10% of reproductive-age women, who present with chronic pelvic pain and/or infertility, leading to negative physical and psychological impact in patient's lives. The pathogenesis involves several hormonal, inflammatory, immune, genetic, and epigenetic mechanisms, which may play a role in the establishment and progression of endometriotic lesions and their clinical manifestations.

In the last years, a possible link between high levels of chronic stress and endometriosis has been hypothesized. Clinical evidence shows that patients with endometriosis report higher levels of perceived stress, anxiety, and depression than healthy women. Stress levels are positively correlated to the severity of the disease and pain intensity. Women with endometriosis, in fact, report impaired quality of life and they are more likely to be affected also by systemic and gynecological comorbidities, contributing to stress in a vicious circle. A high prevalence of immune, inflammatory, allergic and mental health diseases has been found and a strict link with adenomyosis has been shown. Moreover, experimental studies have shown that previous exposure to repeated and excessive stress accelerates the development and increases the severity of endometriosis in animal models and early life stressor contribute to the development of the disease in adult life. However, it is still debatable whether endometriosis is a cause and/or a consequence of stress and which mechanisms explain this relationship.

An adequate management of endometriosis contribute to improve quality of life, reducing stress levels. It has been shown that among women surgically treated, those with worse quality of life had first surgery at a younger age, more symptoms recurrence and more reoperations. Thus, first endometriosis surgery at a later age is an independent predictor of better health condition. Furthermore, patients experiencing repetitive surgery for endometriosis recurrences have lower physical and mental scores than patients receiving a single surgical treatment. Women undergoing hormonal treatment both before and after surgery have a lower rate of recurrence and an improvement of clinical presentation, in terms of pain symptoms. Thus, hormonal treatments represent a valid cornerstone of endometriosis management and may be useful as a first-line therapeutic approach, in order to postpone surgery, and after surgery itself to reduce the risk of recurrence. The gold standard for modern endometriosis management is the individualized approach, aiming to reduce perceived stress, stress-related comorbidities and to improve quality of life.

2. Endometriosis

2.1. Definition and epidemiology

Endometriosis is a chronic disease characterized by the presence of endometrium-like tissue outside the uterine cavity, usually affecting women of reproductive age, typically between 25 and 35 years (Chapron *et al.*, 2019). Endometriosis is estimated to affect 10% of reproductive-age women, however the true prevalence is uncertain, because estimates vary widely among population samples and diagnostic approaches (Zondervan, Becker, Koga, Missmer, Taylor, and Viganò, 2018). The prevalence ranges from 2 to 11% among asymptomatic women, 30-50% among infertile women, and 5 to 21% among women hospitalized for pelvic pain, representing around 176 million women affected worldwide (Buck Louis *et al.*, 2011). Among symptomatic adolescents, it is estimated to reach the 49% for those with chronic pelvic pain and 75% for those with pain that is unresponsive to medical treatment (Janssen *et al.*, 2013).

Endometriosis is a heterogeneous disease with three well- recognized phenotypes: superficial peritoneal endometriosis (SUP), ovarian endometriomas (OMA) and deep infiltrating endometriosis (DIE). In SUP, the least severe form, superficial endometrial lesions occur on the peritoneum, whereas OMA are cystic masses that arise from ectopic endometrial tissue growing within the ovary. DIE phenotype, the most severe, develops deeper than 5 mm under the peritoneal surface (such as the uterosacral ligaments) or infiltrates the muscularis propria of the pelvic organs such as bladder, bowel and ureters (Chapron *et al.*, 2019) (Figure 1). In addition, endometriosis can occur also in extragenital locations, as pleural, diaphragmatic or umbilical (Chamié *et al.*, 2018). In 30% of cases endometriosis is associated to adenomyosis, defined as the infiltration by endometrial stroma and glands into the myometrium. By itself, adenomyosis contributes to pain and infertility commonly observed in endometriosis, and it is specifically characterized by heavy menstrual bleeding (HMB) (Chapron *et al.*, 2020).

The most common presentations of endometriosis include pelvic pain, which involves deep dyspareunia, dysmenorrhea, and noncyclic pain; infertility; dysuria and dyschezia. Unfortunately, these symptoms are not specific to endometriosis and may be signs of other gynecological or non-gynecological conditions, leading to misdiagnosis or significant delay in endometriosis identification (Agarwal *et al.*, 2019). Such painful symptoms may significantly impair physical, mental, and social well-being (De Graaff *et al.*, 2013); and infertility itself may cause psychological stress, low self-esteem, and depression, reducing quality of life (QoL) (Marinho *et al.*, 2018). Furthermore, endometriosis causes a relevant social and economic burden, comparable to other

chronic conditions such as diabetes, Crohn's disease, and rheumatoid arthritis (Soliman *et al.*, 2016).

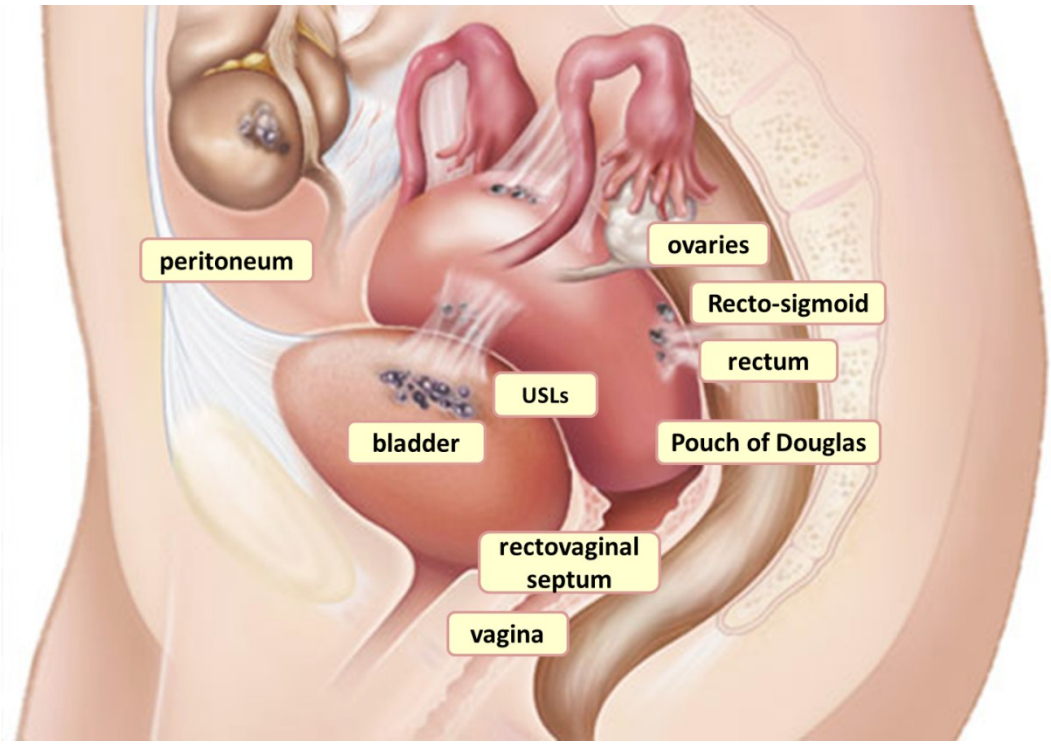


Figure 1. Pelvic endometriosis localizations.

2.2. Pathogenetic mechanisms

Endometriosis is an estrogen-dependent and inflammatory disease (Reis *et al.*, 2013), characterized by progesterone resistance (Reis, Coutinho, Vannuccini, Batteux, *et al.*, 2020). The exact pathophysiology of endometriosis is still unknown. The main hypotheses of the mechanisms implicated in the ectopic location of endometrial cells include retrograde menstruation, coelomic metaplasia, vascular and lymphatic metastatic spread.

The most accepted theory is the retrograde menstruation, according to which menstrual endometrial fragments migrate through the fallopian tubes to the peritoneal cavity, where they are able to implant, develop and sometimes invade other tissues of the pelvis. However, this phenomenon is physiologically observed, thus there are other implicated factors to facilitate this mechanisms. A number of other factors contribute to promote cell survival, proliferation and lesion formation and maintenance, including impaired immunity, neuroangiogenesis, hormonal influences and genetic/epigenetic factors (Patel *et al.*, 2018) (Figure 2). Furthermore, pre-existing endometrial abnormalities might also promote the implantation and growth of pathological endometrial fragments outside the uterine cavity, such as local hyperestrogenism, increased estrogen receptors

activity, progesterone resistance and aromatase overexpression), endometrial neoangiogenesis and a proinflammatory profile in endometrial tissue (Benagiano *et al.*, 2014; Burney and Giudice, 2012; Han and O'Malley, 2014).

The theory that endometriosis arises from cells transdifferentiation from the mesothelium and this process involves reprogramming of multipotent mesenchymal stem cells and support the localization of ectopic endometrial cells at unusual extragenital sites. The same rationale support the theory of lymphatic and vascular dissemination, where endometrial cells are transported through lymphatic channels and veins to colonize distant ectopic sites (Zondervan, Becker, Koga, Missmer, Taylor, and Viganò, 2018).

After migration to ectopic locations, ectopic lesions attach, penetrate and invade: the microenvironment plays a major role to determine maintenance and growth of lesions. Endometriotic lesions undergo cyclic bleeding with repeated tissue injury and repair (Leyendecker *et al.*, 2015), local inflammation, angiogenesis (Filippi *et al.*, 2016) and neurogenesis (Gori *et al.*, 2016). A subsequent cascade of events involving epithelial–mesenchymal transition and fibroblast–myofibroblast transdifferentiation contributes to collagen production and fibrogenesis (Guo, 2018), with entrapment of nerve fibers. This, together with chronic inflammation, worsens pain symptoms.

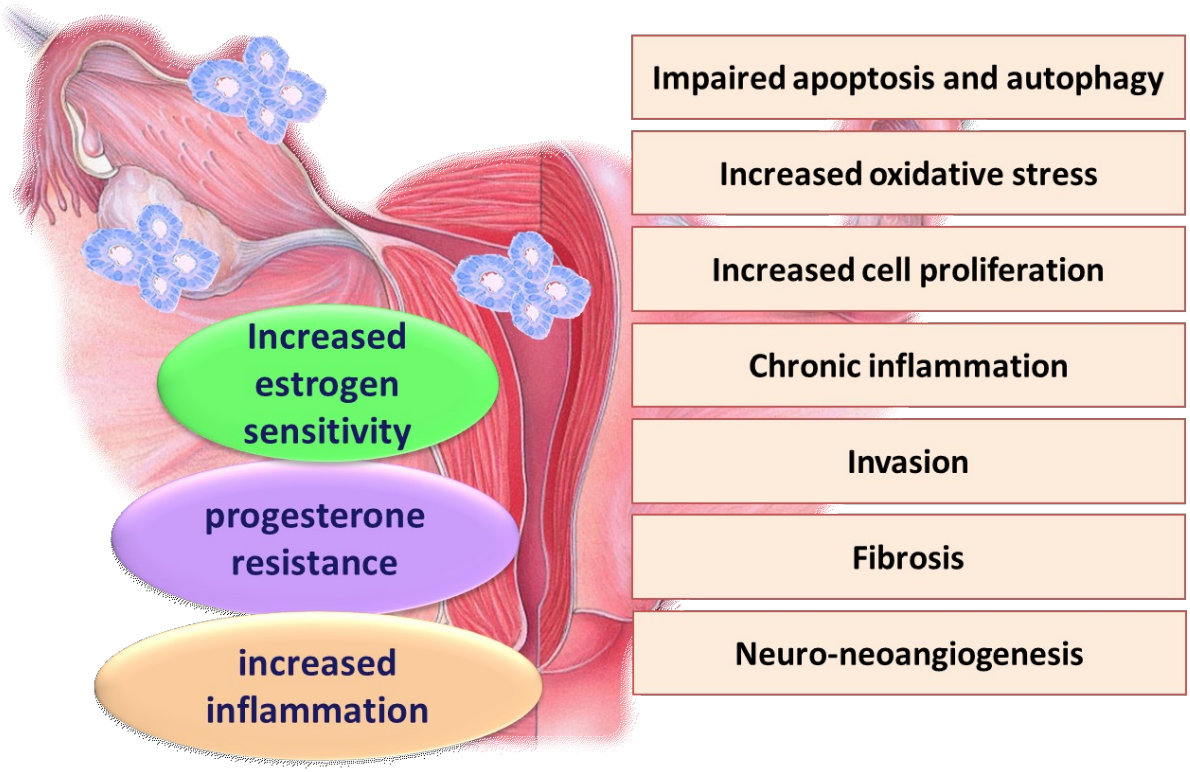


Figure 2. Endometriosis pathogenesis: mechanisms implicated in onset, development and progression of endometriosis lesions

2.3. Clinical presentation: pain and infertility

Endometriosis is a heterogeneous disease also in clinical presentation, as the degree of anatomical involvement does not necessarily correlate to the severity of pelvic pain . Therefore, the stage of endometriosis as classified by the revised American Society for Reproductive Medicine (r-ASRM) is not a good predictor of symptom severity. However, common symptoms include dysmenorrhea and non-menstrual pelvic pain, which can develop into chronic pelvic pain (CPP) (Agarwal *et al.*, 2019). Pelvic pain is insufficient alone as an indicator of endometriosis, as it is associated to a wide variety of gynecological and non-gynecological conditions. In case pain is describe as cyclic, persistent or progressive in menstrual and non-menstrual phase, with a relevant impact on daily life, the likelihood of endometriosis increases dramatically (DiVasta *et al.*, 2018). In some cases, women with endometriosis may remain completely asymptomatic and the diagnosis can be incidentally made during an ultrasound or magnetic resonance exam or during surgery for other reasons. Women with endometriosis are more likely to report also dyspareunia, dyschezia, and dysuria, which usually denote an association with DIE lesions (Fauconnier and Chapron, 2005; Schliep *et al.*, 2015). Although the sensitivity of dyspareunia is generally low, deep dyspareunia is commonly observed in deep locations. Women also frequently report considerable effects on their bowel habits, including alternating constipation and diarrhea, painful emptying of their bowels or blood in the stool (in particular perimenstrually) (Ballard *et al.*, 2010; Schliep *et al.*, 2015). Some women experience recurrent painful urination (dysuria) and/or cyclical blood in the urine (macrohaematuria) and symptomatology related to interstitial cystitis/bladder pain syndrome, which can be associated with endometriosis (Chung *et al.*, 2005). Chest and shoulder pain should be considered suspecting diaphragmatic endometriosis (Redwine, 2002), whereas endometriosis in the ileo-caecal or peri-appendiceal region has been significantly associated to abdominal pain, nausea, vomiting and diarrhea (Fedele *et al.*, 2014). Another frequently present, but often neglected, symptom in women with endometriosis is chronic fatigue, although the exact mechanism remains not fully understood (Álvarez-Salvago *et al.*, 2020).

Another indicator of endometriosis is the response of pain to treatment: from one hand a non-responsive pain to nonsteroidal anti-inflammatory drugs (NSAIDs) should support the suspect of disease, but on the other hand caution is indicated before dismissing NSAID-responsive pain as simply dysmenorrhea (Treatment of pelvic pain associated with endometriosis: A committee opinion, 2014).

Endometriosis is clearly associated with infertility, yet a diagnosis of endometriosis does not always imply infertility. Approximately 30–50% of women with endometriosis have fertility

problems; in parallel, endometriosis is identified in approximately 30% of women in infertile couples (Tomassetti and D'Hooghe, 2018). The disease adversely affects fertility by different mechanisms acting at the level of the pelvic cavity, the ovaries and the uterus (Tanbo and Fedorcsak, 2017). In endometriosis the pelvic cavity becomes an hostile environment because of chronic inflammatory changes in the peritoneal fluid affecting egg quality, folliculogenesis, and luteal function, proliferation of macrophages and phagocytic dysfunction and release of proinflammatory and angiogenic factors. Changes in peritoneal fluid and the distortion of normal anatomy of the fallopian tubes hindering tubo-ovarian contact may affect sperm-oocyte interaction Furthermore, ovaries may present with functional ovarian tissue (ovarian reserve) reduced by endometriomas and/or surgery, leading to decreased ovarian response in assisted reproductive technologies (ART). The chronic inflammatory changes in the pelvis and adhesions may cause ovarian dysfunction. Besides, in endometriosis the uterus itself may show altered endometrial receptivity mainly due to chronic inflammatory changes, to hormonal aberrations and dysperistalsis of the myometrium.

2.3.1. Pain symptoms: peripheral and central mechanisms

Regarding the physiopathology of pain, endometriosis gives rise to nociceptive (including inflammatory), neuropathic and a combination of these (Morotti *et al.*, 2017). In addition, psychological and physical stress and hormonal aberrations influence pain perception (Stratton and Berkley, 2011). The mechanisms that underpin the origin and maintenance of endometriosis-associated pain are related to inflammation, nociception and the interplay between the peripheral and central nervous systems (Brawn *et al.*, 2014; Morotti *et al.*, 2014) (Figure 3). Among peripheral changes in endometriosis-associated pain, neurogenic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), and angiogenic factors are reported to be overexpressed in the peritoneal fluid and in endometriotic lesions of affected women (Barcena de Arellano *et al.*, 2013). Neurotrophic factors are also responsive to estrogens, prostaglandin and cytokine stimulation and sensitize sensory nerve fiber terminals (Krizsan-Agbas *et al.*, 2003). Furthermore, an increased expression of new nerve fibers, a shift in the distribution of sensory and autonomic fibers in some locations, and up-regulation of several neurotrophins have been observed in ectopic endometrium (Tokushige *et al.*, 2006). In DIE, these mechanisms are particularly pronounced, in fact endometriotic lesions and surrounding tissues present higher nerve fibers densities compared with peritoneal lesions and endometriomas (Wang *et al.*, 2009). Moreover, immune mediators (cytokines, interleukins, growth factors) are upregulated in the peritoneal fluid of

women with endometriosis. Histamine, tryptase, serotonin, monocyte chemotactic protein-1 (MCP-1), tumor necrosis factors (TNF), interleukins (IL)-1, -6, and -8, prostaglandins and NGF are abnormally synthesized and released by activated macrophages, mast cells, NK cells and leukocytes within the endometriotic lesions, close to sensory nerve fibers, and in the peritoneal fluid (Riccio *et al.*, 2018). Inflammatory molecules sensitize (lower the threshold) or excite the terminals of sensory nerve fibers, causing the development of a vicious cycle characterized by nociceptor sensitization, local neo-neurogenesis, and activation of sensory nerve fibers, leading to hyperalgesia (Howard, 2009).

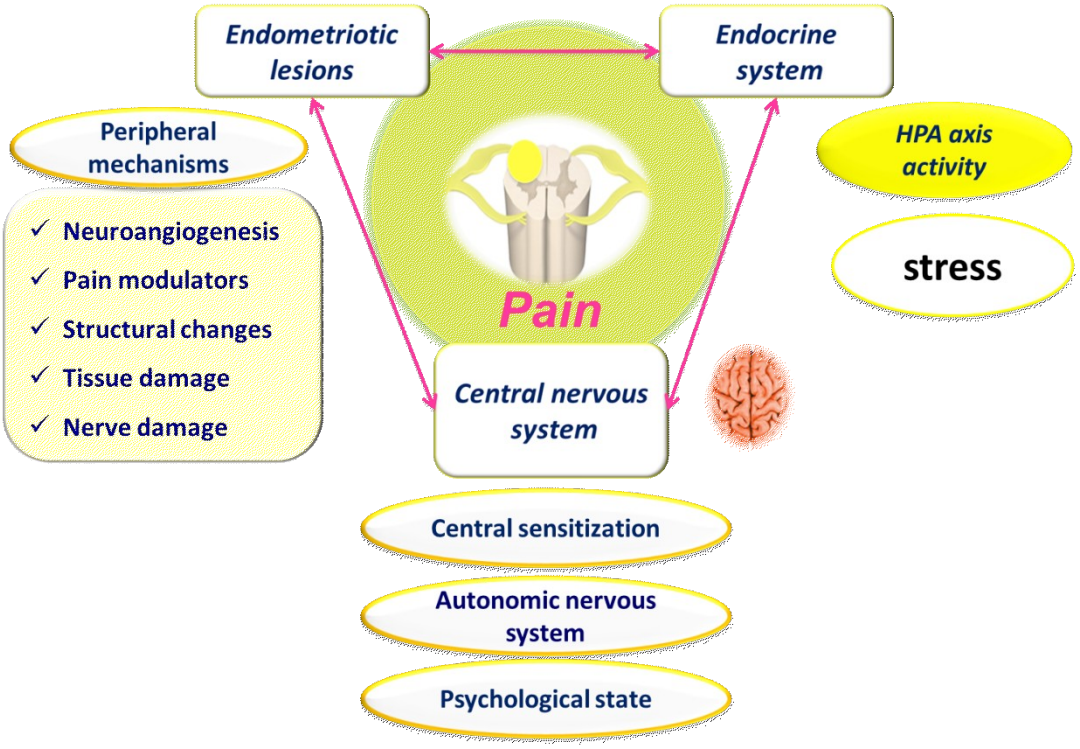


Figure 3: Peripheral and central mechanisms of pain

Endometriotic lesions send noxious signals to dorsal root spinal cord neurons and activate spinal microglia to maintain pain stimuli, resulting in a central sensitization, that represents an enhancement in the function of neurons and circuits in nociceptive pathways caused by increases in membrane excitability and synaptic efficacy (Woolf, 2011). In fact, in endometriosis a number of central changes have been observed: alterations in the behavioral and central response to noxious stimulation, changes in brain structure (both increases and decreases in the volume of specific brain regions), altered activity of both the hypothalamic–pituitary–adrenal axis (HPA) and the autonomic nervous system and psychological distress (Brawn *et al.*, 2014). Regarding changes in structure and

function of Central Nervous System (CNS), women with endometriosis-associated pain showed lower gray matter (GM) volume in brain regions involved in pain transmission (left thalamus, left cingulate gyrus, right putamen, and right insula) and larger GM volume in regions involved in pain modulation and endocrine function regulation (As-Sanie *et al.*, 2012). Women with endometriosis-related CPP show a higher concentration of excitatory neurotransmitters in the anterior insula and a greater intrinsic connectivity between the same cerebral region and the medial prefrontal cortex, supporting a central sensitization (As-Sanie *et al.*, 2016). Similar morphological brain changes have been observed in other recurrent or chronic pain states. Moreover, the presence of central changes has the potential to both exacerbate symptoms and to predispose these women to the development of additional chronic conditions.

2.4 The diagnostic challenge of endometriosis

Despite the increase in awareness among patients and clinicians and the advancement in imaging techniques, endometriosis remains a difficult diagnosis to make (Agarwal *et al.*, 2019). The gold standard for diagnosis is the direct visualization of ectopic endometrial lesions (by laparoscopy) and ideally the histologic confirmation of disease. However, in the last years, imaging techniques - transvaginal ultrasound (TVUS) and magnetic resonance (MRI) – have significantly improved the non-invasive diagnosis of endometriosis (Exacoustos, Manganaro, *et al.*, 2014), overcoming the old concept of "diagnostic laparoscopy". Nowadays, it is generally not necessary to perform invasive surgery only for diagnostic purposes if there is no intention to treat surgically. Thus the diagnostic challenge is due to aspecific symptoms, which are common in other gynecological and non-gynecological diseases, the "normalization" of symptoms, the lack of clinically relevant biomarkers and the aforementioned lack of awareness (Zondervan *et al.*, 2020).

The consequence of such a situation is the diagnostic delay, which ranges from 4 to 11 years from symptoms onset (Ghai *et al.*, 2020). This delay contribute to persistence of symptoms with detrimental impact on quality of life, erosion of the patient-physician relationship, and development of central sensitization, because of disease progression and chronicity (As-Sanie *et al.*, 2019). For health- care professionals, the challenge in daily practice is the determination of whether pain or other symptoms are caused by endometriosis or by other gynecological conditions or syndromes associated with chronic pain, such as irritable bowel syndrome, interstitial cystitis, fibromyalgia and myofascial pain, or by depression and/or a history of sexual abuse. History- taking by patient interviews is essential for diagnosing endometriosis. (Chapron *et al.*, 2019).

Nowadays, imaging is a useful adjunct to clinical diagnostic measures, in fact accuracy improves when it is used adjunctively with symptoms, patient history, and/or physical findings

(Hudelist *et al.*, 2009, 2011). The IDEA consensus statement provides guidance on systematic sonographic evaluation of the pelvis in women with suspected endometriosis (Guerriero *et al.*, 2016). Traditional routine TVS transvaginal ultrasound may be limited to OMA diagnosis; however, "expert-guided" imaging will allow to identify all the phenotypes of pelvic endometriosis also at difficult sites (Bazot *et al.*, 2009; Bazot and Daraï, 2017). TVUS should be the first- line imaging approach for the evaluation of suspected endometriosis. Notably, SUP cannot be visualized by imaging since the size of the lesions is below the threshold for detection. OMAs can be identified reliably by TVUS or MRI with more than 90% sensitivity and specificity. TVUS, when performed by an experienced operator, also has a high sensitivity (91%) and specificity (98%) for detecting and ruling out DIE (Guerriero *et al.*, 2015).

The most appropriate approach to diagnosing endometriosis is based on a combination of patient interviews (Table 1) and clinical examination to enable the selection and identification of patients suspected of having endometriosis. Those selected will undergo imaging, which allows the endometriotic lesion phenotypes (OMA and/or DIE) and possible associated adenomyosis (diffuse and/or focal) to be identified.

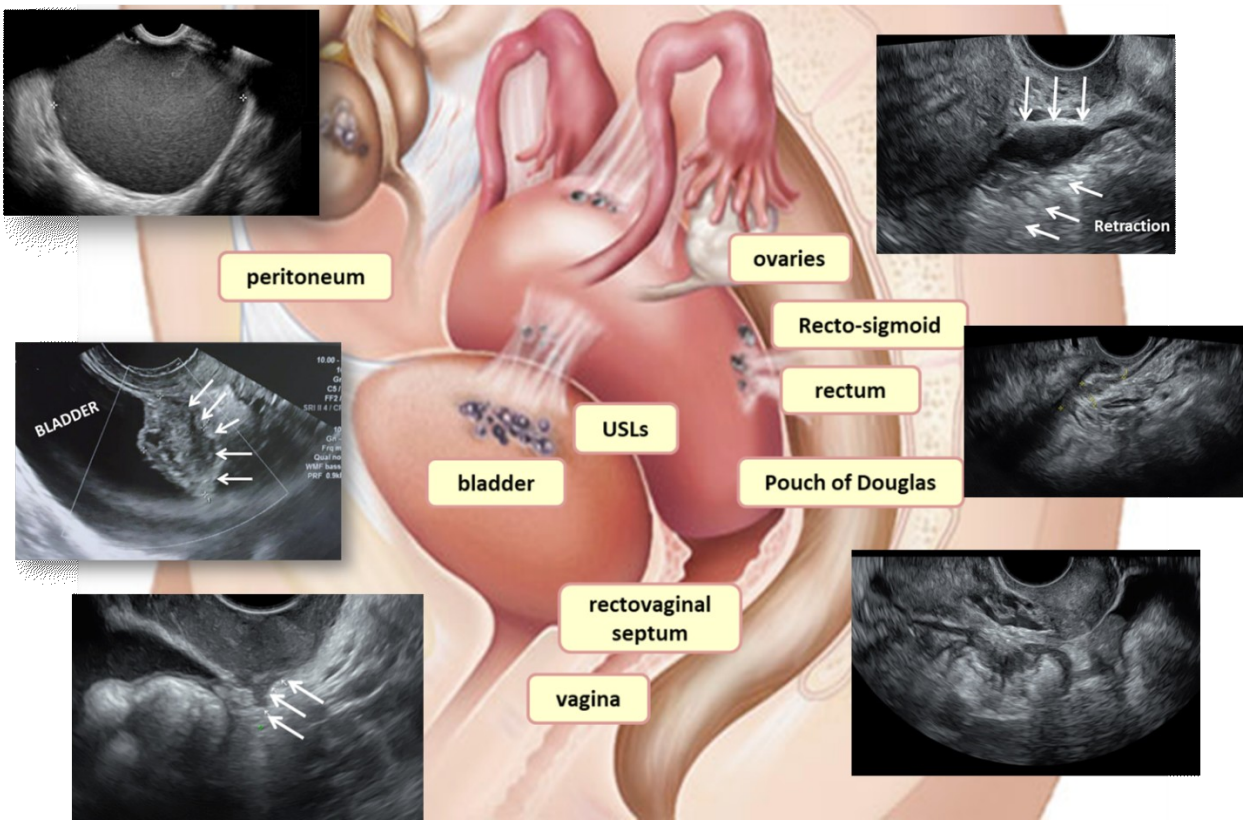


Figure 4. TVUS images of different localizations of endometriosis (from upper left corner clockwise): OMA, recto-sigmoid nodule with fibrotic retraction, bowel nodule, rectal nodule, nodule of the torus, bladder nodule.

Table 1: Risk factors and patient history information to ask for a detailed clinical interview.

Family history of endometriosis
In utero or early childhood factors • Preterm birth • Low birth weight • Neonatal uterine bleeding • Formula feeding • Early- life small body size • Sexual and emotional abuse during childhood251
Adolescent history • Severe primary dysmenorrhea with negative effects on daily life activities • Poor and/or no response to medications used for pain: NSAIDs and/or combined oral contraceptives
Clinical symptoms ✓ Menstrual pain o Persistent and/or worsening cyclic and/or constant pelvic pain o Gastrointestinal pain o Urological pain o Diaphragm pain o Pulmonary pain o Sciatic pain ✓ Infertility ✓ Back pain ✓ Depression ✓ Fatigue ✓ Occupational stress ✓ Insomnia
Associated comorbidities ✓ Autoimmune diseases (systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Sjögren's syndrome, multiple sclerosis and fibromyalgia) ✓ Endocrine diseases (for example, hypothyroidism and Basedow disease) ✓ Asthma, atopic diseases and allergic disorders (hay fever, food allergy and sinus allergic rhinitis) ✓ Migraines ✓ Inflammatory bowel diseases ✓ Cardiovascular diseases (hypertension and hypercholesterolaemia) ✓ Cancer (ovarian, breast or melanoma)
Previous obstetrical history • Adverse pregnancy and perinatal outcomes • Miscarriages
Previous history of pelvic surgery

2.5. The need for a long term management plan

Endometriosis is a chronic disease requiring a lifelong management, with an individualized approach, aiming to reduce pain, perceived stress, stress-related comorbidities and to improve quality of life. Based on patient's symptoms and priorities over the all life, including the desire of pregnancy, a broad- based approach should be used for an adequate management, considering medical treatment, surgery and ART (Chapron *et al.*, 2019; Clemenza *et al.*, 2018).

Until a few years ago, the suspect of endometriosis represented an indication for surgery, mainly used to make the diagnosis through the visualization and histology confirmation of endometriotic lesions. This old concept, given the result of recent literature and recommendations from scientific societies, should be abandoned (Alio *et al.*, 2019). According to the Practice Committee of the ASRM, ''endometriosis should be viewed as a chronic disease that requires a life-long management plan with the goal of maximizing the use of medical treatment and avoiding repeated surgical procedures'(Treatment of pelvic pain associated with endometriosis: A committee opinion, 2014)
Medical treatment can be safely prescribed without histological confirmation of endometriosis (Dunselman *et al.*, 2014; Kuznetsov *et al.*, 2017; Leyland *et al.*, 2010; Practice bulletin no. 114: Management of endometriosis, 2010). As such, in patients who do not have an immediate desire to become pregnant, medical treatment should be the first-line therapeutic option. The use of estroprogestins and progestins should be considered to be "high value care" for women with endometriosis, as they are effective on pain, lesions, and risk of recurrence, in long term management (Vercellini, Buggio, *et al.*, 2016). Overall, these drugs are safe and well tolerated, especially compared with alternate medications for endometriosis, such as GnRH agonists, antagonists, and danazol.

It would be highly desirable to reduce the number of unnecessary and/or inappropriate surgeries for endometriosis, as they entail a risk of recurrence and complications (Ceccaroni *et al.*, 2019; Ianieri *et al.*, 2018; Sibiude *et al.*, 2014) and can negatively affect the ovarian reserve (Goodman *et al.*, 2016). Moreover, ART results in satisfactory fertility outcomes, irrespective of endometriosis phenotype, even without prior surgical removal of OMA and DIE nodules (AlKudmani *et al.*, 2018; Garcia-Velasco and Arici, 2004). The only indications for immediate surgery should be the suspect of cancer in imaging appearance of OMA after imaging, occlusion in bowel DIE and ureteral DIE with ureterohydronephrosis.

In modern endometriosis management, the patient needs to be at the centre of therapeutic decisions. As such, health- care professionals should focus on the patient, in "endometriosis life" view, rather than on the endometriotic lesions themselves (Chapron *et al.*, 2019).

3. Endometriosis and stress

3.1 Stress and the stress mechanisms response

Stress is defined as a threat or an anticipation of a threat to an organism's homeostasis and can be understood as any stimulus, either psychological or physical, capable to promote an unbalance in the body's environment (Selye, 1936). Selye was the first to define stress from a biological point of view as "a nonspecific response of the body to any demand made upon it" (Selye, 1950). The response to stressful stimuli is elaborated and triggered by the stress system, which integrates a wide diversity of brain structures that, collectively, are able to detect events and interpret them as either a real or a potential threat: stressor (Dedovic *et al.*, 2009). The perception of real or potential threats leads to the release of mediating molecules. The interaction between these molecules with their corresponding receptors, in the periphery and in the brain, results in the stress response, which through physiological and behavioral mechanisms restores the body homeostasis and promotes adaptation.

The stress response is complex but has many interconnected mechanisms, including neuroendocrine, inflammatory, and nociception phenomena, with peripheral and central repercussion. Whenever an acute stressful stimulus occurs, an adaptive response is triggered through the activation of two coordinated brain systems: the sympathetic nervous system (SNS) and the hypothalamus-pituitary-adrenal (HPA) axis. At first, the SNS, by releasing catecholamines, promotes an increase in the systemic inflammatory activity. At the level of the hypothalamus, the stress signal activates corticotropin-releasing hormone (CRH) production, leading to the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland, which stimulates the production of glucocorticoids (cortisol) in the adrenal cortex (Figure 5). Cortisol has been shown to influence the activity of the immune system by suppressing cellular differentiation and proliferation, downregulating gene transcription, and reducing cell adhesion. Furthermore, the glucocorticoid production is responsible for counterbalancing the excessive inflammatory response (Godoy *et al.*, 2018). The effects of CRH on the periphery are tissue-specific, having a role in intestinal permeability and motility as well as on uterine function, in addition to mediating behavioral, autonomic, and visceral responses to stress. Ovarian CRH regulates ovarian steroidogenesis, induces the decidualization of endometrial stroma and has a significant role in embryonic implantation and maintenance of pregnancy (Petraglia *et al.*, 2010).

Different types of stressors—physical, emotional, psychological, and social—can activate this axis via different mechanisms, resulting in different kinds of responses (eustress or distress). Normally, the brain responds to stressors by activation of HPA axis, leading to eustress (positive

stress). Chronic activation of the HPA axis by stressors, however, results in distress (negative stress) compromising the immune system, which in turn causes high levels of psychological and emotional distress (Bomholt *et al.*, 2004).

A relationship between stress and disease has been documented for chronic conditions such as cancer, inflammatory bowel disease, and multiple sclerosis, and also for chronic pelvic pain and mood disorders (anxiety, depression, post-traumatic stress syndrome).

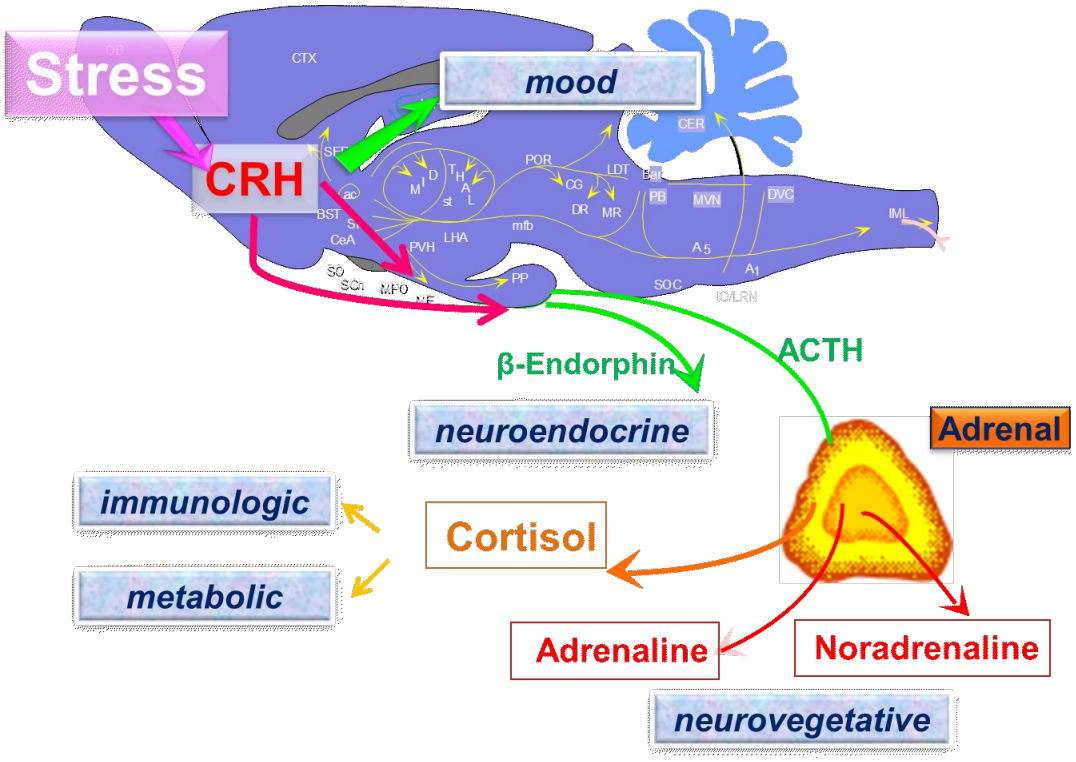


Figure 5. Stress response – HPA activation. The effect of CRH secretion.

3.2. HPA axis in endometriosis

The HPA axis plays a significant role in regulating both the stress signaling and the immune response. On the other hand, the hypothalamic-pituitary-gonadal (HPG) axis is the main system that governs reproductive activities. The crosstalk between the HPA and HPG axes is bi-directional: activation of the HPA axis has suppressive effects in both male and female reproductive activities (Kalantaridou *et al.*, 2010), and the CRH gene is directly regulated by estrogen (Vamvakopoulos and Chrousos, 1993). Therefore, they have effects on regulating endometrial growth and the immune system, so an implication in endometriosis may be hypothesized. Chronic pain can be considered as a repeated stressor, and thus, it is not surprising that dysfunction in the HPA axis is seen in a wide variety of chronic pain conditions, such as endometriosis (Coxon *et al.*, 2018). The mechanisms by which the HPA axis is suppressed in these conditions are not well understood.

Normally, acute stress leads to the activation of the HPA axis and an increase in cortisol levels; however, over time, this response will be attenuated, a condition known as burnout. If this may be a protective mechanisms to reduce continued activation of the body's 'emergency response' systems (Frodl and O'Keane, 2013), in the context of pain, low levels of cortisol may exacerbate painful symptoms by reducing the endogenous analgesia associated with stress (stress-induced analgesia) (Fries *et al.*, 2005).

Few studies have examined the levels of CRH, the CRH receptors, or the associated peptides in women with endometriosis. Higher levels of CRH-binding protein in peritoneal fluid from women with endometriosis than in controls, suggesting possible changes also in circulating levels (Florio *et al.*, 1998). mRNA and the protein of CRH, CRH receptors types 1 and 2, as well as urocortin are more significantly expressed within endometriotic lesions compared with endometrium from healthy women (Carrarelli *et al.*, 2016; Novembri, Borges, *et al.*, 2011; Novembri, Carrarelli, *et al.*, 2011).

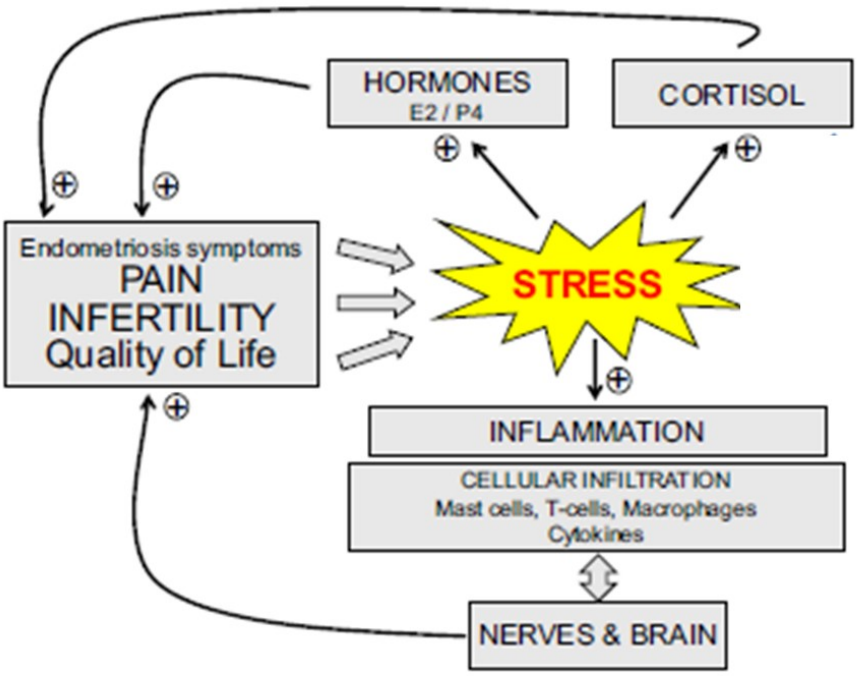


Figure 6. Stress response in endometriiosis via HPA acitivation.

3.3. Is endometriosis a cause of stress?

Endometriosis has two cardinal symptoms: pelvic pain and infertility. Both symptoms per se are capable of evoking a chronic stress response, but their consequences can also be remarkably stressful, giving rise to a vicious cycle. For instance, infertility provokes family demands and fear of

frustrating social expectations (Galhardo *et al.*, 2016; Siedentopf *et al.*, 2008), whereas pelvic pain causes sexual dysfunction and work absenteeism (Soliman, Coyne, *et al.*, 2017), which all may contribute to generate more anxiety and chronic stress. Endometriosis is also surrounded by uncertainty about the disease progression, the success of treatments, the long-term health risks and the reproductive future, which can be additional sources of stress (Harrison *et al.*, 2005; Luisi *et al.*, 2015; Soliman, Coyne, *et al.*, 2017). Furthermore, women with endometriosis experience a delay of 4 to 7 years from first presentation of symptoms to the diagnosis (Soliman, Fuldeore, *et al.*, 2017; Staal *et al.*, 2016), which may further enhance the levels of stress perceived by the patient (Figure 6).

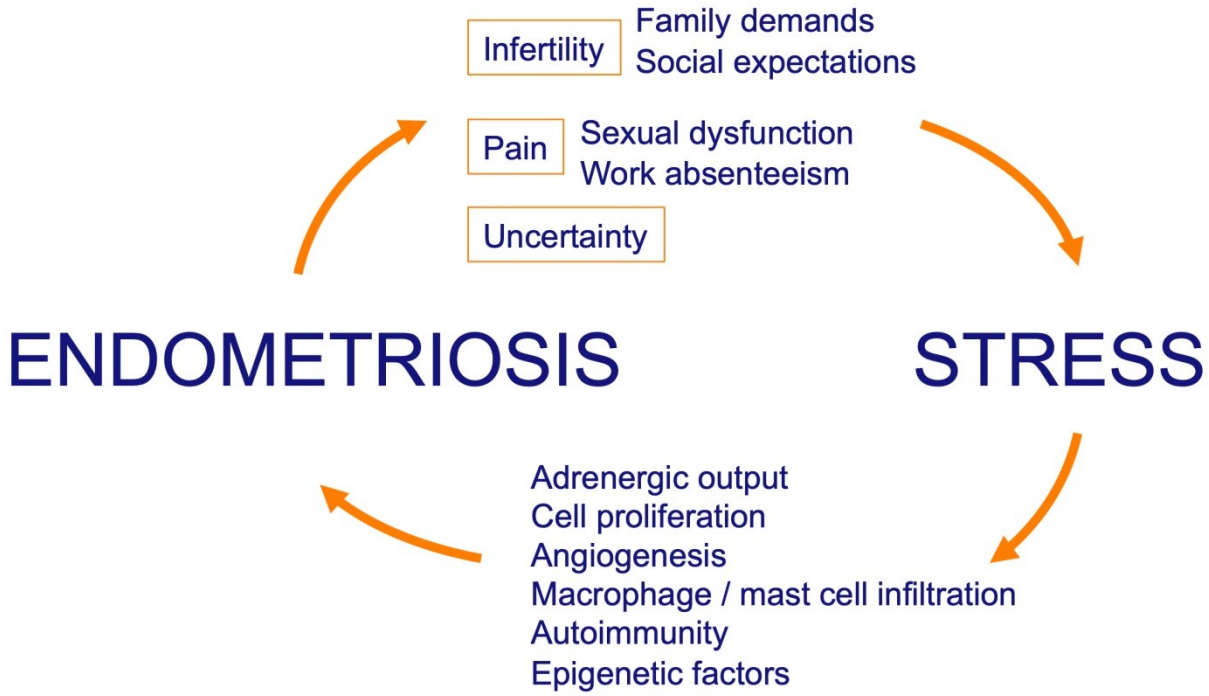


Figure 7. The possible bidirectional cause-effect relation between endometriosis and chronic stress. The figure shows some physical symptoms and social consequences of endometriosis that might induce prolonged stress, and some mechanisms triggered by chronic stress that could boost endometriosis. The arrows highlight the vicious cycle resulting from the association between endometriosis and chronic stress (Reis, Coutinho, Vannuccini, Luisi, *et al.*, 2020).

Objectively assessing the levels of stress that a person is submitted to is not simple, and two main strategies have been used in order to reach this purpose: questionnaires and biomarkers. The first ones can evaluate the frequency and intensity of stress symptoms and the amount of psychological stress perceived. The perceived stress scale (PSS), for example, is a validated psychometric instrument that quantifies the frequency of stressful situations in the last month, in particular those characterized by loss of control, unpredictability, and overload. The stress levels

measured by PSS can be high or very high in more than 70% of women with endometriosis. The stress intensity is highest among women with the most severe disease forms (Lazzeri, Vannuccini, *et al.*, 2015a) and who have been submitted to multiple surgeries (Lazzeri, Vannuccini, *et al.*, 2015b). Stress intensity is higher in women with severe pelvic pain and lower in those who adopt good coping strategies (Zarbo *et al.*, 2018). The application of a therapeutic protocol involving physical and psychological therapy on women with endometriosis and chronic pelvic pain was effective in reducing perceived stress, increasing vitality and improving physical functioning (Friggi Sebe Petrelluzzi *et al.*, 2012).

An indirect measure of stress level is the health-related quality of life (QoL) (Culley *et al.*, 2013) evaluated by using different tools, including the Short Form Health Survey (SF-36 or SF-12, a 36 or 12-item questionnaire which investigates both physical and mental components of health-related QoL) or those specific for endometriosis, such as the Endometriosis Health Profile (Aubry *et al.*, 2017). Endometriosis has a significantly negative impact on health-related QoL scores and the factors involved are mainly linked to pain symptoms (Facchin *et al.*, 2015). A recent study by Marki et al. reported that both physical pain symptoms and emotional regulation difficulties, the latter being mediated by psychological stress, had a negative impact on health-related QoL of women with endometriosis (Márki *et al.*, 2017). In addition, other factors contribute to the psychological health and stress perception of women with endometriosis, such as self-esteem, body esteem and emotional self-efficacy (Facchin *et al.*, 2017).

The second way to assess the levels of stress is by measuring the products of the stress response cascade. Among the stress biomarkers usually assessed in clinical studies, the most characteristic one is cortisol. In fact, a prolonged and intense stress stimulus may disrupt the HPA axis and alter the normal pattern of cortisol release. Interestingly, the deregulation of the HPA axis can culminate in either an over response (i.e., hypercortisolism) or a paradoxical phenomenon of adrenal fatigue leading to hypocortisolism (Blackburn-Munro and Blackburn-Munro, 2003). In fact, incongruent patterns of cortisol alterations have been observed in women with endometriosis.

Higher hair cortisol levels were found in patients with endometriosis than in healthy women of similar age, parity, education level and body mass index (BMI) (van Aken *et al.*, 2018). Similarly, increased serum cortisol levels were detected in infertile women with endometriosis compared to fertile healthy women and the highest levels were found in those with advanced stage endometriosis (Lima *et al.*, 2006). Interestingly, physical and psychological interventions have been shown to normalize salivary cortisol levels of women with endometriosis and chronic pain(Friggi Sebe Petrelluzzi *et al.*, 2012). On the other hand, some studies have observed the opposite trend (Petrelluzzi *et al.*, 2008; Quiñones *et al.*, 2015). Petrelluzi et al. showed that patients with

endometriosis and chronic pelvic pain had low concentrations of salivary cortisol, measured in three samples collected 8 hours apart, and a high level of perceived stress, associated with a poor quality of life (Petrelluzzi *et al.*, 2008). Quinones et al. observed among patients with endometriosis an association of salivary hypocortisolism and infertility and dyspareunia, but not dysmenorrhea (Quiñones *et al.*, 2015).

The use of cortisol as a stress biomarker has some limitations. The normal release of this glucocorticoid obeys circadian and ultradian pulsatile rhythms, with a wide variation in pulse frequency and amplitude, and can be influenced by external factors. Such variability renders difficult the long-term analysis of cortisol concentrations and the differentiation between acute and chronic stress by using plasma, urine or saliva samples. Cortisol concentration in the scalp hair, in its turn, provides an average measure of free cortisol levels over a period of time (Lee *et al.*, 2015) and hence it seems to be more appropriate for measuring chronic stress (Greff *et al.*, 2019).

Another marker of chronic stress is salivary alpha-amylase, which reflects the adrenergic axis of the stress response (Lynch *et al.*, 2014; Nater and Rohleder, 2009), we are not aware of any study on salivary alpha-amylase levels in women with endometriosis. Serum levels of acute stress hormones, such as prolactin and urocortin-1, are elevated in women with endometriosis (Bilibio *et al.*, 2014; Maia *et al.*, 2018), but no relationship with pain intensity or level of stress response has been evaluated so far in such patients.

Altogether, the available evidence from clinical studies clearly indicates that endometriosis is a condition associated with high levels of stress. Although it is possible to induce from data available that the disease may cause chronic physical and psychological stress, most studies were cross sectional and thus had no possibility to demonstrate a temporal relationship between the stress response and the evolution of endometriosis. However, the evidence that the stress intensity assessed by PSS decreased one month after the surgical treatment of moderate to severe endometriosis suggests that treating the disease contributed to reduce the stress levels of the patients (Lazzeri, Vannuccini, *et al.*, 2015b).

3.4. The impact of stress in endometriosis onset and development

The second vector of the equation endometriosis-stress is the possibility that the stress response affects the evolution of endometriosis. Investigating this cause-effect relation is not an easy task because it would require leaving symptomatic patients without treatment. Current diagnostic methods do not provide any screening test for initial endometriosis and therefore render virtually impossible to investigate in humans whether stress is a risk factor for the establishment of

endometriotic implants. Despite the lack of any clinical trial that evaluated a direct effect of chronic stress on the onset, progression and invasiveness of endometriotic lesions, recent studies allow us to hypothesize that such relation might exist.

A retrospective case-control study (Vannuccini *et al.*, 2016) showed that mothers of women with endometriosis were significantly more likely to have smoked during the patient's gestation, increasing the risk of antenatal hypoxemia. In addition, those affected reported more frequently perinatal complications during their gestation, such as prematurity, lower birth weight and preeclampsia (Borghese *et al.*, 2015; Upson *et al.*, 2015; Vannuccini *et al.*, 2016). We therefore hypothesize that the intrauterine and neonatal exposure to prolonged physical stress stimuli could be linked to the future development of endometriosis. In our opinion, the intrinsic mechanisms involved in this process might be diverse from those implicated in adulthood. While in adults the chronic stress response might directly enhance the progression of endometriotic lesions, early in life the remodeling of neurobiological systems responsible for the stress response (Gunnar and Quevedo, 2007) might induce epigenetic factors predisposing the future onset of the disease.

If chronic stressful events in the perinatal period may increase the risk of endometriosis in the future, we should next consider the same hypothesis regarding stressful events in childhood. In effect, negligence and abuse during childhood may evoke persistent changes in neural and neuroendocrine systems and consequently hyperactivity of the HPA axis. The combination of precocious exposure to adversity and the presence of a genetic predisposition to anxious personality could produce a personal tendency to have exacerbated stress responses to external stimuli (Fuentes and Christianson, 2018; Tafet and Nemeroff, 2016). Recently, a prospective cohort study showed that the risk of endometriosis was greater among women with history of severe physical abuse or severe sexual abuse when compared with those not reporting any previous maltreatment. In particular, the risk of laparoscopically-confirmed endometriosis increases up to 79% for women reporting severe-chronic abuse of multiple types (Harris *et al.*, 2018). Similarly, results from a case-control study showed that childhood sexual abuse, emotional abuse/neglect and inconsistency experiences were associated with the diagnosis of endometriosis, suggesting the importance of early stress exposure (Liebermann *et al.*, 2018).

Furthermore, small body size/low BMI in childhood and adolescence has been linked on one side with chronic stress (Lo Sauro *et al.*, 2008) and on the other side with the risk of endometriosis (Shah *et al.*, 2013; Vitonis *et al.*, 2010). In fact, endometriosis was reported to be more common among women who were leaner at 8 years, at menarche and at 20-25 years (Farland *et al.*, 2017). The body size during childhood seems to be so important that those who were the smallest size between 5 and 10 years have an 18% increased risk to develop endometriosis later in life (Vitonis *et*

al., 2010). In addition, an inverse correlation has been shown between BMI during early adulthood and endometriosis development: young women who were underweight had a relative risk of 1.31 to be affected by the disease (Shah *et al.*, 2013). Moreover, patients with the lowest BMI (<18.5) had the highest risk to develop more severe phenotypes, such as deep infiltrating endometriosis (Lafay Pillet *et al.*, 2012).

Nevertheless, the association between low BMI and endometriosis remains unexplained (Figure 8). An enhanced activity of HPA axis has been demonstrated in patients with anorexia nervosa and in high performance athletes, inducing a condition of chronic stress (Lo Sauro *et al.*, 2008). Strenuous physical activity has been reported to increase by 16% the risk of endometriosis (Vitonis *et al.*, 2009), while leisure activity seems to decrease this risk to some extent (Garavaglia *et al.*, 2014). Thus, stress stimuli, linked to dietary restriction or high intensity physical activity, seem to facilitate endometriosis development. Experimentally, Goetz et al. have demonstrated in mice that endometriosis induces weight loss by disrupting hepatic metabolism (Goetz *et al.*, 2016). They showed a higher expression of four genes related to an anorexigenic effect and a reduced expression of two genes associated with obesity and metabolic disease in mice with induced endometriosis. Surprisingly, caloric restriction promotes autophagy and blocks the lesion growth in this animal model (Yin *et al.*, 2018).

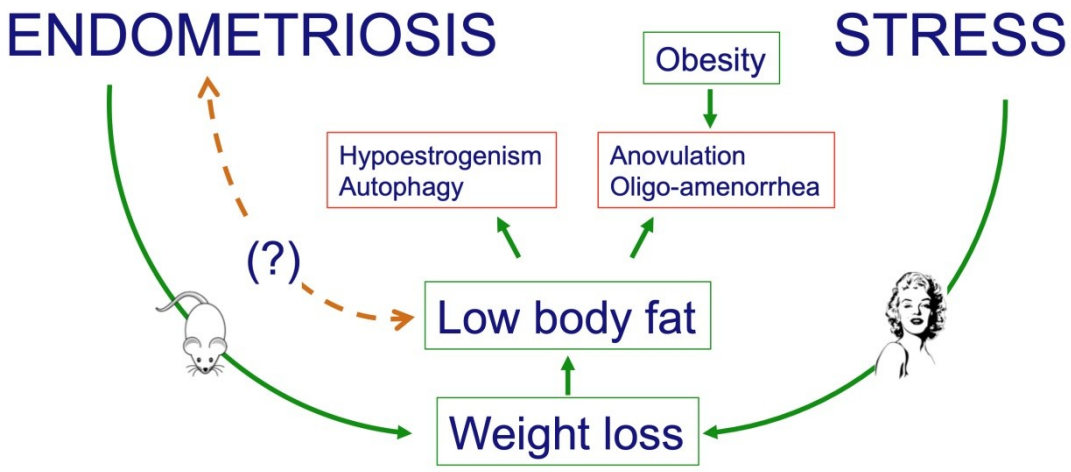


Figure 8. Weight loss is associated with stress (in certain clinical conditions) and with endometriosis onset (in animal model). However, the association between low body fat and endometriosis remains unexplained. The known reproductive consequences of weight loss (framed in red) are theoretically protective against endometriosis, but, paradoxically, epidemiological data link low body fat to increased endometriosis risk (Reis, Coutinho, Vannuccini, Luisi, *et al.*, 2020).

A number of inflammatory and immune-related conditions have been linked to high levels of perceived stress, while the occurrence of early stressful events and many of them seem to be

related to endometriosis as well (Jones, 2016; Kvaskoff *et al.*, 2015). A Danish nationwide cohort study showed that women with endometriosis have an increased risk of inflammatory bowel diseases, such as Chron's disease and ulcerative colitis, even 20 years after diagnosis (Jess *et al.*, 2012). Women with endometriosis have also high risk to develop allergic manifestations, such as hay fever, sinus allergic rhinitis, and food allergy (Bungum *et al.*, 2014). Furthermore, autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, multiple sclerosis, fibromyalgia and chronic fatigue syndrome, are more likely to be diagnosed in women with endometriosis (Caserta *et al.*, 2016; Greenbaum *et al.*, 2019; Harris *et al.*, 2016; Nielsen *et al.*, 2011).

Distress and pain symptoms have a negative impact on each other, leading to the so-called 'sickness response', associated with a vicious cycle caused by the underlying neuroendocrine–immune imbalance (Tariverdian *et al.*, 2007). Chronic fatigue syndrome, fibromyalgia, asthma and rheumatoid arthritis have been associated with hypocortisolism, probably due to receptor resistance mechanisms (Heim *et al.*, 2000). Other chronic pelvic pain syndromes such as inflammatory bowel disease and interstitial cystitis seem to cause a hyperactivity of the HPA axis, despite the limited data available (Bernstein, 2017; Videlock *et al.*, 2009). Enhanced cortisol levels have been described, as well, in syndromic mood and anxiety disorders (Chrousos, 2009).

The chronic pain experienced by patients with endometriosis and other chronic pain disorders might have a psychosomatic component, beyond physical and biomolecular causes. In fact, although endometriosis severity does not correlate with pelvic pain intensity, stress probably does (Martin *et al.*, 2011). According to a recent cross-sectional study, more than half of interviewed women with endometriosis resulted positive to mental health disorders as assessed by the Patient Health Questionnaire, whose results strongly correlated with pain severity: women with severe pain had multiple psychiatric diseases and the somatoform disorder was the most common (Vannuccini, Lazzeri, *et al.*, 2017). Furthermore, anxiety and depression traits, and a higher tendency of pain catastrophizing are commonly present in endometriosis patients and can amplify the perception of pain (Laganà *et al.*, 2017; McPeak *et al.*, 2018; Rocha *et al.*, 2017). It is possible that such relationship could lead to the above cited 'sickness response', contributing to the evolution of the disease.

Studies using animal models of endometriosis have also been used to shed light onto the possible effect of stress in the course of endometriosis. Female rats exposed to the stress model of forced swimming during 10 days and a control group without stress were subsequently grafted with autologous uterine fragments to induce peritoneal endometriosis (Cuevas *et al.*, 2012). Compared to the non-stressed group, the rats that had been previously exposed to swimming stress developed

larger and more numerous endometriotic lesions. When applied two weeks after the induction of endometriosis, swimming stress also accelerated the growth of endometriotic implants and mast cell infiltration in the implant area, besides increasing the expression of nerve growth factor in the remaining uterine horn (Cuevas *et al.*, 2018). Moreover, when the animal was given the possibility of "controlling" the swimming stress by floating on a platform, the endometriosis lesions evolved similarly to those of non-stressed controls (Appleyard *et al.*, 2015). This suggests that the level of stress controllability appears to modulate the behavior and pathophysiology of endometriosis, offering evidence for potential therapeutic interventions.

Another series of experiments by Guo and coworkers tested the effects of psychological stressors on the development of endometriosis in mouse model. Exposure to a predator outside the mouse's cage for 24 hours every other day for two weeks after inducing endometriosis caused a worsening in the evolution of the disease, characterized by faster lesion growth, macrophage infiltration and increased angiogenesis (Guo *et al.*, 2017). Immobilization stress, either before or after endometriosis induction, was able to boost lesion growth and local angiogenesis, an effect that was prevented by treatment with propranonol, indicating that beta-adrenergic input to the endometriotic lesions was involved in the deleterious effects of the stress (Long *et al.*, 2016). Another study on psychological stress and pain perception in mouse model of endometriosis showed that animals subjected to water avoidance stress for 7 days developed more severe symptoms, but interestingly stress reversed the allodynic effect caused by endometriosis, maybe due to the stress-induced analgesia phenomenon (Hernandez *et al.*, 2017).

Women with endometriosis have increased stress, as indicated by psychological and endocrine stressmeasures, and the stress intensity correlates with pain severity and disease extension. On the other hand, chronic stress may represent a primary cause of endometriosis, and, therefore, avoiding or treating chronic stress might potentially reduce the risk of developing endometriosis. Furthermore, perinatal and childhood stress should be considered as risk factors for endometriosis.
However, many questions remain to be addressed, in order to clarify the causal link between endometriosis and stress and to assess whether stress-reducing therapies are effective to mitigate symptoms and/or slow down the development of endometriotic lesions.

4. Endometriosis and impact on women's health

4.2 Endometriosis and systemic comorbidities

4.2.1 Research study 1

Comorbidities and quality of life in women undergoing first surgery for endometriosis: differences between Chinese and Italian population

Aim of the study

Debilitating pain-related symptoms (dysmenorrhea, dyspareunia, dysuria, dyschezia) and infertility are commonly observed in women with endometriosis. A negative association exists between endometriosis-associated pain and daily activities, self-care and productivity at work up to job loss and these may cause a low quality of life (Lazzeri, Orlandini, *et al.*, 2015). Medical or surgical treatment of endometriosis aim to achieve a complete and durable symptom relief, with good physical and mental health (Vannuccini *et al.*, 2019), however, the disease is chronic and recurrent (Guo, 2009).

Furthermore, endometriosis is associated with gynecological (Capezzuoli *et al.*, 2020) and systemic comorbidities, including immune (asthma, rheumatoid arthritis, psoriasis, and multiple sclerosis), inflammatory (bowel inflammatory disease, Crohn's disease) and psychiatric disorders (depression and anxiety) (Kvaskoff *et al.*, 2014; Surrey *et al.*, 2018; Vannuccini *et al.*, 2018).

The majority of epidemiological data on endometriosis comes from reports on Caucasian women (Bougie, Healey, *et al.*, 2019). However, also Asian women are likely to be diagnosed with endometriosis, whereas African-American women are less frequently affected (Fawole *et al.*, 2015; Flores *et al.*, 2008). Few evidences are available on the Chinese population (Yen *et al.*, 2019), but there is a high number of patients with endometriosis in China because of the large population base. Moreover, information on women with endometriosis differentiated by ethnicity are quite limited (Bougie, Yap, *et al.*, 2019). The present study aims to evaluate the difference between Chinese and Italian population with endometriosis in terms of symptoms, comorbidities and Quality of Life (QoL).

Materials and Methods

An observational cross-sectional multicenter study was conducted in a group (n=371) of fertile age women (25-45 years old) with endometriosis, recruited in four different hospitals, all third level centers for endometriosis treatment (Florence and Negrar di Valpolicella, Verona, Italy; Shanghai

and Guangdong, China). They were divided into two age-matched groups based on the ethnicity: a) Chinese population (n=175) and b) Italian population (n=196). We included only women with histological diagnosis of endometriosis Data were collected within two years after a single surgery for endometriosis through a structured questionnaire, during a clinical follow up visit. Women with previous or actual pregnancy were excluded. Also women with multiple surgical interventions for endometriosis were not included in the study. A database was built collecting for each case all the following information:

a) demographic characteristics (age, body mass index (BMI), age at menarche);
b) endometriosis data: age at the diagnosis of endometriosis, phenotype of endometriosis at surgery (OMA, DIE, SUP, mixed phenotype), if hormonal medical treatment before and surgery was performed, and current symptoms (dysmenorrhea, dyspareunia, non-menstrual pelvic pain and urinary pain), measured as absent, mild, moderate and severe, according to the visual analog scale (VAS). In fact, women graded their perception of each type of pain on a 10-cm line from 0 (no pain) to 10 (unbearable pain); a mean VAS score of 7 or higher was considered severe;
c) gynecological (uterine fibroids, adenomyosis, polycystic ovarian syndrome) and systemic comorbidities, including autoimmune (thyroiditis, rheumatoid arthritis, psoriasis, pemphigus, multiple sclerosis, myasthenia gravis), metabolic (obesity, hypertension, hypercholesterolemia), inflammatory (allergic rhinitis, allergic asthma, irritable bowel syndrome, inflammatory bowel diseases) and mental health diseases (depression, anxiety, panic disorder, eating disorders); it has been also asked whether the disorder appears before or after the diagnosis of endometriosis;
d) evaluation of health-related Quality of Life (QoL) by using the Short Form-12 (SF-12) (Gandek *et al.*, 1998).

The data were validated through review of medical records of all participants to confirm what was reported by patients, especially for systemic comorbidities. The study was approved by the locals Institutional Review Boards and all participants provided written informed consent to be included in the series.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software, version 22 (IBM Corporation, Armonk, NY, USA). Statistically significant differences between groups were determined using Student's *t*-test for continuous variables and Chi-square test or Fisher's exact test for categorical variables. A p value <0.05 was considered statistically significant. The sample size was estimated to detect differences of at least 0.5 standard deviations in quantitative variables or

20% in the frequency of categorical variables between groups with 80% statistical power and 95% confidence level. In order to achieve a statistical power of 95%, we needed at least 111 patients with a significance level of 0.05 and an effect size of 0.3.

Results

The group of Italian women resulted younger than the Chinese at diagnosis of endometriosis (P<0.0001). Chinese women were more frequently diagnosed with OMA (84.0% vs 46.7%) whereas Italians had significantly more DIE (4.1% vs 1.8%) and mixed phenotypes (40% vs 5.6%) (p<0.0001) (Table 1). Furthermore, the group of Italian women underwent more frequently to medical hormonal treatment before surgery (21% vs 43.3%, p<0.0001). On the contrary, no differences were found in terms of indications for surgery, which was mainly represented by pain symptoms (Table 1).

Regarding gynecological comorbidities, PCOS (7.9% vs 5.3%) and uterine fibroids (9.3% vs 3.2%) were more common in the Chinese than in Italian patients, while adenomyosis was more common in Italians (27.9% vs 9.9%). Most of the systemic comorbidities observed were more common in Italian women than in the Chinese, i.e. autoimmune diseases (27.5% vs 4.6%, p<0.0001), metabolic/endocrine diseases (14.8% vs 8.7%, p=0.007), inflammatory diseases (44.5% vs 19.9%, p<0.0001) and mental health disorders (35.3% vs 3.7%, p<0.0001) (Table 2). Among the comorbid autoimmune diseases the Italian population showed more thyroiditis (17.9% vs 3.4%), dermatological diseases (psoriasis, pemphigus) (4.1% vs 0) and neuromyopathies (multiple sclerosis, myasthenia gravis) (2.6% vs 0) than the Chinese population. Similarly, some of the comorbid metabolic/endocrine diseases were more common in Italian patients including hypertension (7.7% vs 1.7%) and obesity (3.6% vs 0), as well as some inflammatory diseases, i.e. allergic asthma (1.5% vs 0), intestinal inflammatory diseases (3.6% vs 0). Also mental health disorders were more common in Italian patients, i.e. depression (19.4% vs 1.1%), anxiety (10.7% vs 1.7%) and anorexia (1.5% vs 0). Whether the comorbidities were identified before or after the diagnosis of endometriosis was also evaluated and Italian women have already been diagnosed with a systemic disease before endometriosis identification (Table 2).

No differences were found in terms of medical treatment after surgery between the two groups (60.2% vs 78.2%, p=0.3904). Regarding pain symptoms at the follow up visit, Italian patients suffered more frequently from severe pain than the Chinese, in terms of dysmenorrhea (p<0.0001), dyspareunia (p<0.0001), non-menstrual pelvic pain (p<0.0001) and dysuria (p=0.011) (Table 1). Accordingly, the evaluation of QoL in the two groups showed that the Chinese patients had both for SF-12 physical score (46.5±8.7 vs 41.5±9.9, p<0.0001) and SF-12 mental score (47.1±8.8 vs

37.2±10.7, p<0.0001) higher than the Italian patients (Figure 1). Stratifying patients according to endometriosis phenotypes, those with DIE among Italians have significantly worse mental scores compared to Chinese (36.8±10.4 vs 49.8±8.2, p=0.010), whereas physical scores were not significantly different (41.9±7.3 vs 44.6±13.1, p=0.649), although the sample size in Chinese population is very small. Regarding women with only ovarian localization of endometriosis among Italians and Chinese, we confirmed that Italians reported more severe pain symptoms (dysmenorrhea 26.7% vs 10.5%; dyspareunia 13.5% vs 1%; non menstrual pelvic pain 16.5% vs 0%) and a worse QoL (SF-12 physical score 41.3±11.8 vs 46.7±8.5, p=0.010; SF-12 mental score 38.5±10.9 vs 47.4±9.0).

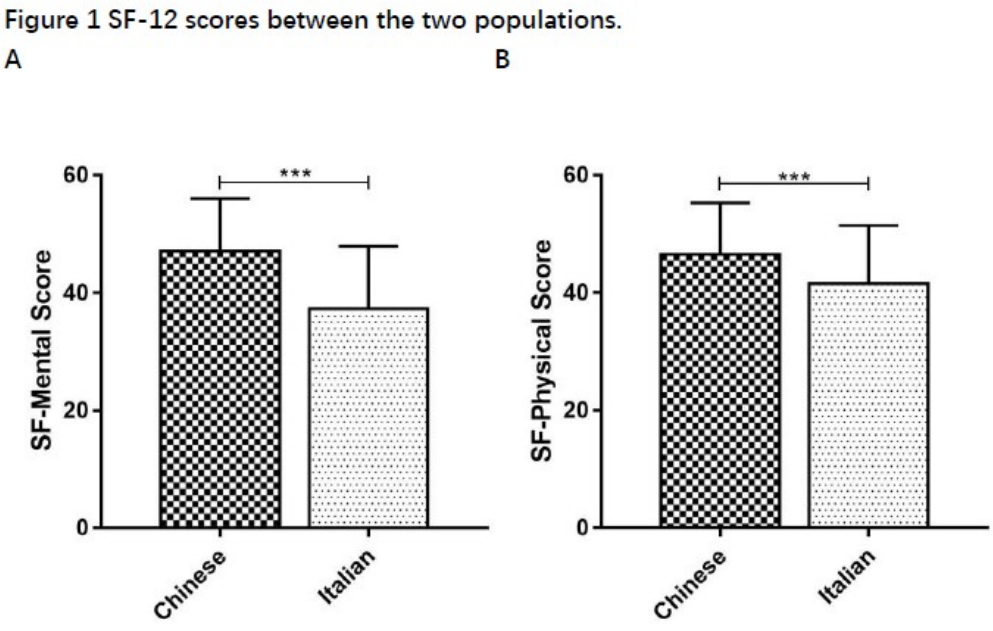
Table 1 Clinical characteristics of the two populations. Values are mean+SD or n (%).
BMI: Body Mass Index; OMA: ovarian endometriosis; DIE: Deep Infiltrating Endometriosis; SUP: superficial endometriosis.

	Chinese (n=175)	**Italian (n=196)**	**P-value**
Age (years)	36.5±6.7	36.3±6.4	0.769
BMI	21.4±3.1	22.2±3.3	0.021
Menarche (years)	13.5±1.6	12.3±1.3	<0.0001
Age at 1st diagnosis (years)	32.2±5.9	27.3±5.6	<0.0001
Medical treatment before 1st surgery	34 (21.0%)	85 (43.4%)	<.0001
Indication for 1st surgery			
Pain	92 (54.8%)	104 (53.1%)	.025
Pain and infertility	7 (4.2%)	24 (12.2%)	
Infertility	8 (4.8%)	4 (2%)	
Imaging	61 (36.3%)	64 (32.7%)	
Endometriosis phenotype at surgery			
OMA	137 (84.0%)	91 (46.7%)	<0.0001
DIE	3 (1.8%)	8 (4.1%)	
SUP	14 (8.6%)	14 (7.2%)	
OMA+DIE	4 (2.5%)	26 (13.3%)	
OMA+DIE+SUP	0(0.0%)	25 (12.8%)	
OMA+SUP	5 (3.1%)	24 (12.3%)	
DIE+SUP	0(0.0%)	7 (3.6%)	
Dysmenorrhea			
No	52 (36.6%)	60 (31.7%)	<0.0001
Mild	56 (39.4%)	34 (18.0%)	
Moderate	14 (9.9%)	52 (27.5%)	
Severe	20 (14.1%)	43 (22.8%)	
Dyspareunia			
No	94 (76.4%)	57 (29.7%)	<0.0001
Mild	24 (19.5%)	43 (22.4%)	
Moderate	3 (2.4%)	66 (34.4%)	
Severe	2 (1.6%)	26 (13.5%)	
Non menstrual pelvic pain			
No	92 (66.2%)	57 (29.5%)	<0.0001
Mild	35 (25.2%)	50 (25.9%)	
Moderate	10 (7.2%)	56 (29.0%)	
Severe	2 (1.4%)	30 (15.5%)	
Urinary pain			
No	123 (88.5%)	142 (74.0%)	0.011
Mild	11 (7.9%)	32 (16.7%)	
Moderate	5 (3.6%)	16 (8.3%)	
Severe	0(0.0%)	2 (1.0%)	
Medical treatment after surgery	130 (60.9%)	154 (78.2%)	0.3904

Table 2 Gynecological and systemic comorbidities of endoemtriosis in the two populations. Values are mean+SD or n (%). PCOS: polycystic ovary syndrome.

	Chinese (n=175)	Italian (n=196)	P-value
Gynecological comorbidities			
No	104 (68.9%)	115 (60.5%)	<0.0001
PCOS	12 (7.9%)	10 (5.3%)	
Adenomyosis	15 (9.9%)	53 (27.9%)	
Uterine fibroids	14 (9.3%)	6 (3.2%)	
Multiple	6 (4.0%)	6 (3.2%)	
Before endometriosis diagnosis	43/65 (91.5%)	3/25(4.0%)	<0.0001
Autoimmune diseases	8 (4.6%)	51 (26.0%)	<0.0001
Before endometriosis diagnosis	1/7 (14.3%)	36/51 (70.6%)	0.007
Metabolic/endocrine diseases	28 (8.7%)	39 (14.8%)	0.007
Before endometriosis diagnosis	2/13 (15.4%)	14/26 (53.8%)	0.023
Inflammatory diseases	46 (19.9%)	95 (44.5%)	<0.0001
Before endometriosis diagnosis	10/16 (62.5%)	68/78 (87.2%)	0.027
Mental health disorders	5 (3.7%)	77 (35.3%)	<0.0001
Before endometriosis diagnosis	1/5 (20.0%)	45/65 (69.2%)	0.044

Figure 1. The histograms showed the SF-12 physical and mental scores in Chinese and Italian population respectively. ***P<0.001.



Discussion

The present cross-sectional and multicentric study explored for the first time the difference in endometriosis phenotypes, comorbidities and QoL between Italian and Chinese patients with history of a single surgery for endometriosis. The Italian endometriotic patients showed more frequently DIE and mixed phenotypes and systemic and gynecological comorbidities than the Chinese patients. In addition, the Italian group presented at the follow up visit within 2 years after surgery with lower SF-12 physical and mental scores and more severe pain symptoms after stratifying for endometriosis phenotype and despite no differences in terms of post-surgery hormonal medical treatment.

Generally, endometriosis has been considered as a disease of Caucasian and middle-class women who delayed children-bearing and, at the beginning, the majority of the studies were performed in Caucasian population. In the last years, a number of studies have been published on the Chinese population and differences in genetic mutations that predispose Asian and Western populations to endometriosis have been found (Dai *et al.*, 2018). Furthermore, genome wide association studies reported that among North Chinese polymorphisms in rs12700667 located within the intergenic region of 7p15 are associated especially with an elevated risk of OMA, which is consistent with the higher incidence of OMA found in our study (Li *et al.*, 2017). On the contrary, the Italian group showed more frequently DIE and mixed phenotypes, which is consistent with worse pain symptoms and lower QoL scores (Koninckx *et al.*, 2012; Montanari *et al.*, 2019). We may hypothesize that this finding may be due to a more accurate pre-operative imaging diagnosis, which contributes to the laparoscopic identification of deep lesions and to the adequate site-mapping of endometriosis (Exacoustos, Malzoni, *et al.*, 2014). A number of Italian gynecologist devoted to endometriosis management are themselves experts in imaging for endometriosis, which improves the pre-operative diagnosis, especially of DIE lesions (Exacoustos *et al.*, 2017). This difference may account for an underestimation of DIE in the Chinese. However, the higher incidence of OMA among Chinese is in line with previous reports (Hu *et al.*, 2012), and it is also consistent with their better QoL, given the lower association of this phenotype with pain symptoms, if DIE is not associated (Chapron *et al.*, 2012). Conversely, the DIE phenotype is more frequently linked to a more severe and aggressive clinical presentation, due to specific pathogenic mechanisms such as a highly decreased apoptosis, an increased proliferation activity related to oxidative stress, a higher expression of metalloproteinases and activins for invasiveness and a relevant activation of neuroangiogenesis in ectopic endometrial lesions, compared to other phenotypes (Tosti *et al.*, 2015).

Furthermore, the two groups have significantly different baseline characteristics, such as younger age at diagnosis and more comorbidities in Italian women. No differences were found in terms of indications for surgery, with a similar rate of pain symptoms or imaging findings (i.e. adnexal mass) among the two groups. In addition, the study involved only referral hospitals for endometriosis with expertise in both surgical and medical treatment of the disease. Their protocols for endometriosis management were similar, following international guidelines (Alio *et al.*, 2019; Dunselman *et al.*, 2014): depending on age, pregnancy desire, pain symptoms, endometriosis phenotype and each woman's wishes, an individualized management was planned. Younger age at surgery may denote, on one hand, a more aggressive presentation of the disease, whereas on the other it may contribute to more severe symptoms and worse QoL in Italian women with endometriosis (Lazzeri, Vannuccini, *et al.*, 2015a). A more relevant impact of endometriosis in Italian than Chinese women may be also explained by the difference in the medical approach, in the access to health care, in the acceptance to medical treatment between the two populations. Our study showed that Chinese underwent less frequently to a pre-operative medical hormonal treatment, whereas the rate of post-surgical hormonal treatment was approximately the same. Given the low pain scores and the better quality of life in Chinese compared to Italian group in our study, a potential role played by the Traditional Chinese Medicine (TCM) should be considered. TCM is commonly used in China to provide pain relief control, the recurrence of endometriosis following surgery, and to improve QoL (Su *et al.*, 2014; Zhao, Hao, *et al.*, 2013; Zhao, Liu, *et al.*, 2013; Zhu *et al.*, 2014). TCM users were also less likely to require surgical treatment for endometriosis than non-users (Su *et al.*, 2014) and among those operated was effective in controlling recurrences (Zhao, Hao, *et al.*, 2013; Zhao, Liu, *et al.*, 2013). However, although TCM is often used for the management of endometriosis patients, there is a lack of high quality clinical evidence supporting its effectiveness compared to other medical treatments. However, as data on the use of TCM missing in our data, its potential role in contributing to pain control and to postpone first surgical intervention should be taken into account.

In other multicenter studies it was shown that ethnicity influenced the access to health care, diagnosis and treatment for endometriosis (Bougie, Yap, *et al.*, 2019), thus, as a exposure factor, ethnicity throws its effect on clinical presentation and management through social and cultural constructs rather than genetics (Yudell *et al.*, 2016). Various components of pelvic pain are the primary symptoms of endometriosis and several studies support that individual conceptualization of pain may be affected by different social cultures in different races (Frisch, 2014; Kwok and Bhuvanakrishna, 2014; Yudell *et al.*, 2016). Asian patients tend to normalize pain, while Caucasian patients more likely to seek health care positively (Campbell and Edwards, 2012). A cross-country

study reported that the incidence and the intensity of pain symptoms were significantly lower in Chinese population than Russia and France (Chapron *et al.*, 2016). In our study, Italian patients with endometriosis suffered more from severe pelvic pain than Chinese women, suggesting a different cultural background rather than actual pain conceptualization. Also the different health system organization, with less coverage for outpatient clinic and primary care in China determining a less request for medical care, entails a diverse approach to such a chronic and multifaceted disease.

Furthermore, Italian women presented more commonly with systemic comorbidities, especially inflammatory diseases and mental health disorders (such as depression and anxiety). On the contrary, in the Chinese population, the most common comorbidities were gynecological (PCOS, uterine fibroids). Despite the prevalence of comorbidities resulted significantly different between the two groups, the diagnostic criteria of each disorders was well defined according to the current and updated guidelines, which stands for all over the world. Furthermore, the systemic comorbidity reported by the patient during medical history collection was always confirmed by reviewing medical records. Regarding the potential differences between Italy and China overall in terms of incidence of comorbidities, according to the current relevant literature there are no significant differences between the two population in terms of baseline incidence of autoimmune, inflammatory and metabolic diseases (Li *et al.*, 2019; Ng *et al.*, 2013). Besides, data on comorbidities among Italians are consistent with figures reported in already published studies on endometriosis (Bungum *et al.*, 2014; Chiaffarino *et al.*, 2020; Kvaskoff *et al.*, 2014; Parazzini *et al.*, 2017; Shigesi *et al.*, 2019). The different distribution of comorbidities with endometriosis in the two population suggested a different spectrum of diseases under different society and culture. A better identification of systemic comorbidities should be taken into account in Italian group, because of the presence of a multidisciplinary team - including an immunologist, a gastroenterologist, a psychiatrist, a dietician, a neurologist, a pain specialist and a physical therapist - in the Italian Endometriosis centers. However, figures from Italians are absolutely in line with those reported in studies on cohorts from all over the world on women with endometriosis. A better investigation on systemic comorbidities among Chinese may be desirable in order to identify potential diseases accompanying endometriosis, in order to better explain clinical presentation and to plan a fully comprehensive treatment (Teng *et al.*, 2016). This would also allow to increase the awareness of endometriosis among specialists of other disciplines. Moreover, these comorbidities were mostly diagnosed before endometriosis, suggesting a complex inner correlation between a number of systemic diseases and predisposition to endometriosis development. However, given the cross-

sectional study design and a short time period of observation since the first operation for endometriosis, it is not possible to account for potential comorbidities would have developed later.

SF-12 is a validated instrument for quantifying the Health-Related Quality Of Life (HRQOL) and higher scores on the SF-12 physical component summary (PCS) and mental component summary (MCS) indicate better quality of life. The SF-12 scores of PCS and MCS were significantly higher in Chinese than in Italian patients with endometriosis, suggesting that the QoL in the Chinese patients resulted better than in the Italian patients and this does not depend on the phenotype of endometriosis. In fact, after stratifying for DIE and OMA phenotype, data on pain symptoms intensity and QoL among Chinese women were still better than in the Italian Group. As already mentioned, a potential role of TCM should be considered, even though no data are available on that variable. Probably, the better scores among Chinese patients are in part related to the lower number of comorbidities in the Chinese women, suggesting a possible poor attention to this kind of questioning when clinical history was collected. A study reported that QoL was independently associated with more severe dysmenorrhea and more severe chronic pelvic pain, but not with higher ASRM stage (Facchin *et al.*, 2015; McPeak *et al.*, 2018). A correlation between different phenotypes of endometriosis and levels of stress perception was observed (Lazzeri, Orlandini, *et al.*, 2015; Luisi *et al.*, 2015), suggesting a possible association between the forms of endometriosis and impact on QoL. Besides, the presence of psychiatric diseases was related to endometriosis-associated pain but not with lesions localization (Eriksen *et al.*, 2008; Sepulcri Rde, 2009). It is clear that the adverse effect of endometriosis on QoL are related to various factors, including pain/discomfort symptoms, infertility and high intensity of stress perception (Lazzeri, Orlandini, *et al.*, 2015; Nnoaham *et al.*, 2011a; Reis, Coutinho, Vannuccini, Luisi, *et al.*, 2020). In addition, ethnicity may affect quality of life of endometriotic patients through different pain conceptualization and access and acceptance to health care.

Some limitations and strengths of the study should be acknowledged. The study included only women with endometriosis who underwent first surgery in referral centers for endometriosis. Currently a large number of patients with endometriosis are only medically treated with good results and the first-line approach for endometriosis symptoms should be medical treatment (Vercellini *et al.*, 2018). However, the study is focused only on those with a previous surgical operation for endometriosis, so our results cannot be applied to all endometriosis-affected women. The choice of including only women with histological diagnosis of endometriosis aimed to have strict criteria of inclusion for the centers involved in the research. in order to minimize bias due to different imaging techniques and no universally shared diagnostic criteria for the non-invasive diagnosis of each phenotype of endometriosis. Women with history of multiple surgery were

excluded, in order to reduce the impairment of QoL due to repeated surgery, relapses and recurrences. In addition, as the study is cross-sectional it is not possible to predict which comorbidities would have developed as time passes.

Based on the results, in young women with comorbid diseases and mild symptoms, the possibility of endometriosis should be considered in order to not delay the diagnosis of endometriosis.

Moreover, more and more patients with endometriosis-associated pain required a better treatment of the various comorbidities in order to improve their quality of life.

4.3 Endometriosis and gynecological comorbidities

4.3.1 Research study 2

Ultrasound findings in infertile women with endometriosis: evidence of concomitant uterine disorders

Aim of the study

Epidemiological data show that endometriosis is observed in 50% of infertile women (Tomassetti and D'Hooghe, 2018), but no data exist on the coexistence of adenomyosis and uterine disorders in those patients. Adenomyosis is the presence of endometrial glands and stroma into the myometrium, causing pain and abnormal uterine bleeding (Chapron *et al.*, 2020; Vannuccini and Petraglia, 2019). Data obtained from surgical dataset showed a prevalence of adenomyosis in women with endometriosis ranging between 20% and 80% (Chapron *et al.*, 2017; Di Donato *et al.*, 2014; Lazzeri *et al.*, 2014). These data were collected pre-operatively by TVUS or MRI. No study investigated the prevalence of adenomyosis in women with endometriosis, referred to infertility clinics, independently from the surgical treatment.

Similarly, imaging studies are available on the prevalence of uterine fibroids in endometriosis, originating from surgical and histological reports in perimenopausal women. Uimari et al (Uimari *et al.*, 2011) 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 an hysterectomy for benign uterine disorders had endometriosis and adenomyosis in 40.4%, endometriosis and uterine fibroids in 22.7%, both conditions in 34.1% (Naphatthalung and Cheewadhanaraks, 2012). In a similar report on women undergoing surgery for benign gynaecologic disease, the coexistence of endometriosis with uterine fibroids, adenomyosis, and benign ovarian cysts were 28%, 43.5%, and 50%, respectively (Tanmahasamut *et al.*, 2014). Coexisting uterine fibroids and endometriosis were identified in 21.2% of patients undergoing laparoscopy myomectomy (Maclaran *et al.*, 2014), but no data are available on the sonographic association between endometriosis and uterine fibroids in infertility clinics.

The aim of the present study was to evaluate the sonographic prevalence of adenomyosis and uterine fibroids in patients with endometriosis at different age intervals in an infertility center.

Materials And Methods

A retrospective cohort study was performed on women with clinical or ultrasound suspect of endometriosis, referred from 2015 to 2018 to our Endometriosis outpatient clinic during the infertility work-up (Careggi, University Hospital, Florence, Italy). They underwent a further TVUS evaluation performed by expert sonographers (n=586). The included cases were infertile women in reproductive age (25-52 years old) with ultrasound evidence of endometriosis lesions. Clinical and US reports and images were reviewed and an electronic database was built.

Ultrasonography was performed by using two ultrasound machines (Voluson E8, GE) and a transvaginal probe (5-7.5 MHz) (RIC 6-12-D, GE) by three gynaecologists with high level expertise on gynaecological US. During the examination, uterus, adnexa and pelvic compartments (anterior, posterior and recto-vaginal) were assessed. Presence and localization of endometriosis (OMA and DIE), as well as the presence of uterine fibroids or adenomyosis, were evaluated and described according to IDEA (International Deep Endometriosis Analysis) (Guerriero *et al.*, 2016), MUSA (Morphological Uterus Sonographic Assessment) (Van Den Bosch *et al.*, 2015) and IOTA (International Ovarian Tumor Analysis) consensus (Van Holsbeke *et al.*, 2010). Those are statements on terms, definitions and measurements that may be used to describe and report the sonographic features of endometriosis, myometrium and ovarian masses, respectively.

OMA lesions were described according to IOTA criteria, identifying the ultrasound-homogeneous 'tissue' with homogeneously dispersed echogenic cystic contents ('ground glass' appearance) (Van Holsbeke *et al.*, 2010). DIE lesions were described according to IDEA criteria (Guerriero *et al.*, 2016). Anterior compartment DIE ultrasound appearance included hypoechoic linear or spherical lesions, with or without regular contours involving the muscolaris or submucosa of the bladder. In the posterior compartment DIE lesions can appear as hypoechoic thickening of the wall of the bowel or vagina or ad hypoecoic solid nodules with smooth or irregular contours.

Uterine fibroids and adenomyosis were described according to MUSA criteria (Van Den Bosch *et al.*, 2015). Ultrasound appearance of fibroids is typically described as a well-defined round lesion within the myometrium or attached to it, often showing shadows at the edge of the lesion and/or internal fan-shaped. On color- or power-Doppler imaging, circumferential flow around the lesion is often visible. Size, localization and types of uterine fibroids were described.

Adenomyosis was described as diffuse, if present in the myometrium as dispersed, or focal, if forming a confined lesion. In case it was present as a large cyst a cystic adenomyosis was identified. Both 2-dimensional (2D) and 3-dimensional (3D) features of adenomyosis were used: enlarged uterus with myometrial anteroposterior asymmetry; junctional zone (JZ) thickened (regular or irregular) and/or interrupted; myometrial lesions with ill-defined outline, shape and/or contour;

no rim; no edge shadows or fan-shaped shadowing; non uniform/mixed echogenicity with cyst and translesional vascular flow (Van Den Bosch *et al.*, 2015).

A total number of 419 patients with US confirmed endometriosis were included in the study. Histological confirmation of endometriosis was not considered an inclusion criterion to the study, only a small percentage of women had history of previous surgery for endometriosis (12%), as most of women were a first referral for a clinical or sonographic suspect of endometriosis. The flowchart of study population and the subgroups according to endometriosis phenotypes are shown in Figure 1. We described the uterine disorders characteristics and the presence of gynaecological comorbidities in Table 1. The ultrasound coexistence with uterine fibroids and/or adenomyosis was investigated and the study group was subdivided in 3 groups of patients according to age (<35years; 35≥years>45; ≥45 years). In particular we evaluated whether the prevalence of uterine fibroids and adenomyosis was different in the three subgroups and whether age influenced the coexistence of endometriosis and uterine disorders. Moreover we compared the endometriosis phenotype prevalence (OMA, DIE or both) in the three subgroups. Finally, we analysed if a particular endometriosis phenotype (OMA, DIE or both) was associated with uterine fibroids and adenomyosis, independently of age. We have not evaluated the influence of lesions size on the variables analysed because, with the exception of OMA, the measurements are not standardized and poorly reproducible. Similarly, the reported history of multiple and temporally heterogeneous medical treatments did not allow to obtain reliable results on the influence of previous interventions on uterine comorbidities prevalence. The study follows the principles of the Declaration of Helsinki. Participants gave written informed consent for participating in the study. Continuous and binomial variables were collected and an electronic database was analysed by using SPSS (SPSS 22.0 version). ANOVA test and χ^2 test were used as appropriate. Significance level was stated at <0.05

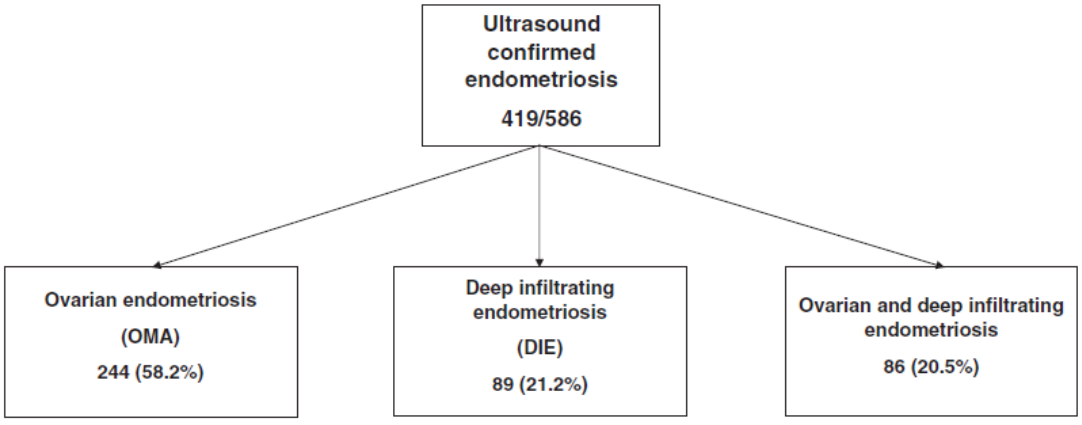


Figure 1. Flow chart with of study population.

Results

US prevalence of uterine disorders in patients with endometriosis is shown in Table 1. In these patients, uterine fibroids were present in 3.1% of cases, whereas adenomyosis in 21.2%. The co-existence of fibroids and adenomyosis was reported in the 14.6% of patients.

The majority of fibroids were intramural (11.7%), whereas submucous fibroids were the rarest (1.2%), Subserous myomas were 8.6% of the total. Uterine congenital malformations were present in only 5 patients (1.2%).

Comparing the prevalence of gynecological comorbidities according to age, patients aged>35 years were more likely to be affected by uterine fibroids (p=0.003), adenomyosis (p=0.030) or both uterine fibroids and adenomyosis (p<0.0001). No statistically significant association was found between endometriosis phenotypes and uterine disorders.

Furthermore, no association was observed between patients' age and endometriosis phenotype (Table 2 and Table 3).

Table 1. Gynecological comorbidities in patients with ultrasound diagnosis of endometriosis.

Characteristics	
Mean age (years)	35.6 ± 8.0
Uterine disorders comorbidities	
a. Uterine fibroids	13/419 (3.1%)
b. Adenomyosis	89/419 (21.2%)
c. Both fibroids and adenomyosis	61/419 (14.6%)
Type of uterine fibroids	
a. Submucous	5 (1.2%)
b. Intramural	49 (11.7%)
c. Subserous	36 (8.6%)
Uterine congenital malformations	
a. Yes	5/419 (1.2%)
b. No	414/419 (98.8%)
Polycystic ovary ultrasound appearance	
c. Yes	39/419 (9.3%)
d. No	380/419 (90.7%)

Table 2. Uterine disorders in women with endometriosis according to age intervals.

	Age <35 years $n = 192$ (45.8%)	Age 35 ≥ and < 45 years $n = 183$ (43.7%)	Age ≥ 45 years $n = 44$ (10.5%)	*p* value
Uterine congenital malformations	4 (2.1%)	1 (0.5%)	0 (0%)	.291
Endometriosis phenotypes				
OMA	108 (56.2%)	113 (61.7%)	23 (52.3%)	.390
DIE	39 (20.3%)	40 (21.9%)	10 (22.7%)	.905
Both OMA and DIE	45 (23.4%)	30 (16.4%)	11 (25%)	.178
Uterine disorders comorbidities				
Uterine fibroids	0 (0%)	11 (6%)	2 (4.5%)	.003
Adenomyosis	30 (15.6%)	49 (26.8%)	10 (22.7%)	.030
Both uterine fibroids and adenomyosis	13 (6.8%)	34 (18.6%)	14 (31.8%)	<.0001

Table 3. Uterine disorders in women with endometriosis according to phenotype.

	Ovarian endometriosis (OMA) $n = 244$ (58.2%)	Deep infiltrating endometriosis (DIE) $n = 89$ (21.2%)	Both OMA and DIE $n = 86$ (20.5%)	p value
Uterine congenital malformations	3 (1.2%)	1 (1.1%)	1 (1.2%)	.996
Uterine disorders comorbidities				
Uterine fibroids	7 (2.9%)	2 (2.2%)	4 (4.7%)	.623
Adenomyosis	58 (23.8%)	14 (15.7%)	17 (19.8%)	.264
Both uterine fibroids and adenomyosis	38 (15.6%)	14 (15.7%)	9 (10.5%)	.482

Discussion

The present study evaluated the sonographic coexistence of uterine disorders (adenomyosis and uterine fibroids) in patients with endometriosis referred for infertility. We have observed that patients aged>35 years were more likely to be affected by uterine fibroids, adenomyosis or both disorders.

TVUS allows the non-invasive diagnosis of adenomyosis, whereas in the past the diagnosis was made on histological examination after hysterectomy (Vannuccini and Petraglia, 2019). In addition, TVUS is a relatively accessible imaging modality and it has an increasing role in the management of patients with adenomyosis (Andres *et al.*, 2018). TVUS is the first level method to diagnose also uterine fibroids. In the 2015, the MUSA group described the features that can be used in the differential diagnosis between the two conditions (Van Den Bosch *et al.*, 2015).

Adenomyosis is often associated with pelvic endometriosis and seems to be more frequent in severe forms of disease. In fact, a recent study on pre-surgical US evaluation of reproductive age women before undergoing laparoscopic surgery for pelvic pain described a strong association between uterine adenomyosis and stage IV endometriosis (Dior *et al.*, 2019). Similar to previous studies (Di Donato *et al.*, 2014), we found that a prevalence of isolated adenomyosis in patients with pelvic endometriosis was 21.2%. The association between uterine fibroids and endometriosis is less clear, even if the histological prevalence of uterine fibroids in women with endometriosis ranges between 20% and 30% (Naphatthalung and Cheewadhanaraks, 2012; Tanmahasamut *et al.*, 2014).

The majority of studies evaluating the association between uterine disorders and endometriosis are based on pre-surgical assessment of patients with pelvic endometriosis or uterine fibroids. Concerning adenomyosis, 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 DIE (Di Donato *et al.*, 2014, 2015).

A higher prevalence of adenomyosis was found by Eisemberg et al, who observed a 89.4% prevalence of US signs of adenomyosis in women with history of surgery of endometriosis (Eisenberg *et al.*, 2017). On the contrary, Lazzeri et al found a 47.8% prevalence of adenomyosis in women with DIE, influencing significantly the pre- and post-surgical dysmenorrhea severity (Lazzeri *et al.*, 2014). A similar prevalence of adenomyosis (59.9%) was detected by using MRI in symptomatic women younger than 42 years, undergoing surgery for benign gynecological pathologies (Chapron *et al.*, 2017). Finally, 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 to an older age, higher gravidity and parity and presence of pelvic endometriosis (Naftalin *et al.*, 2012).

Considering uterine fibroids, Ujmari et al showed a 25.8% prevalence in patients operated for endometriosis, while in patients undergoing surgery for fibroids the prevalence of endometriosis was 19.6% [9]. A modest further increased prevalence of endometriosis in surgery for fibroids was detected by Tanamhasut (28%) (Tanmahasamut *et al.*, 2014). Endometriosis was more common in those with subfertility and less common in those with bleeding disorders (Maclaran *et al.*, 2014).

Endometriosis, uterine fibroids and adenomyosis can affect fertility in different ways. Endometriosis related infertility is associated to ovarian damage and alteration of pelvic cavity due to inflammation and adhesions with distortion of pelvic architecture, inflammatory changes in peritoneal fluid and altered endometrium (De Ziegler *et al.*, 2010).

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 life of the disease (Tomassetti and D'Hooghe, 2018).

Adenomyosis can causes infertility by causing aberrant uterine contractility, abnormal myometrial activity and deranged endometrial milieu with altered expression of implantation factors (Vannuccini, Tosti, *et al.*, 2017). Adenomyosis seems to affect fertility in a very strong way even in the absence of endometriosis, as described by a pioneer study in baboons (Barrier *et al.*, 2004) and then confirmed in subsequent reports in humans, even though the level of evidence and the epidemiological available data are still not strong enough to draw firm conclusions (Soliman, Fuldeore, *et al.*, 2017; Tomassetti *et al.*, 2013).

Uterine fibroid are present in 5–10% of infertile women, but they represent the unique cause of infertility only in 2-3% (Vlahos *et al.*, 2017). These data suggest that other mechanisms, such as endometriosis, can interfere with fertility in women with uterine fibroids. Uterine fibroids may

determine distortion of the uterine cavity, alteration to the endometrial and myometrial blood supply, deviation or obstruction of the tubal ostia, alteration of the tubo-ovarian anatomic relation, chronic endometrial inflammation, impairing implantation (Zepiridis *et al.*, 2016). Considering the relevance of endometriosis in affecting fertility, the coexistence of other uterine disorders would interfere and possibly worsen the chance of conception, especially in those requiring ART. The understanding of concomitant gynecological conditions affecting fertility may allow a more comprehensive counselling and a better plan for fertility desire. The identification of coexistent uterine fibroids or adenomyosis allows to plan a pretreatment, either medical or surgical, before ART.

Some limitations need to be acknowledged, as the study has a retrospective design and there are no controls. However, the research is one of the few US reports on the prevalence of coexistent uterine disorders in endometriosis, considering also fibroids. The results provide an epidemiological overview in infertile women with endometriosis. In addition, all the US have been performed by expert sonographer, using the same terminology and features to define a diagnosis.

In conclusion, we would like to reiterate the importance of US assessment in the evaluation of endometriosis, recommending to pay particular attention to eventually associated uterine disorders, such as uterine fibroids and adenomyosis, for a better management of the patient. This is crucial in the infertility clinic, where a global evaluation determines the choice of the correct treatment for conception and favorable pregnancy outcome. Moreover, the coexistence of endometriosis and uterine disorders may have significant implications for patient care and consequent medical and/or surgical treatment, independently from the desire to conceive.

TVUS is the first line imaging technique for the evaluation of suspected endometriosis and associated uterine disorders (uterine fibroids and adenomyosis). In fact, it permits the characterization of OMA and DIE lesions, as well as adenomyosis and uterine fibroids, driving the clinician to the correct approach. One of the main limit of TVUS is that SUP cannot be visualized. In case of diagnostic uncertainty, the prescription of continuous hormonal treatment can be used as clinical test, avoiding unnecessary diagnostic laparoscopies. In infertility management, TVUS is necessary in the choice of the correct and patient-oriented treatment, considering endometriosis, uterine disorders and other gynecological comorbidities. This diagnostic techniques helps the clinician in the selection of surgical or ART approach, considering an huge amount of variables, for example the endometriosis phenotype and pelvic anatomy, the ovarian reserve, the presence of fibroids distorting the uterine cavity and many others (Chapron *et al.*, 2019; Muzii *et al.*, 2017, 2020).

Personalized treatment is fundamental and the correct therapy is not the same for every patient, above all in a complex disease such as endometriosis.

4.4 Endometriosis and quality of life

Quality of life (QoL) is defined as a multi-dimensional construct of the individual perception of one's position in life in the context of culture and value systems in relation to goals, expectations, standards, and concerns (The World Health Organization quality of life assessment (WHOQOL): Position paper from the World Health Organization, 1995). It is affected in a complex way by the person's physical health, psychological state, level of independence, social relationships, beliefs. Endometriosis has been reported to deteriorate multiple domains in women's lives, including daily activities, social relationships, family planning and work productivity (Culley *et al.*, 2013).

The SF-36, a questionnaire for general QoL, consists of 36 items in eight domains: physical functioning; role-physical; bodily pain; general health; vitality; social functioning; emotional; and mental health. It has been validated in women with endometriosis and is an useful tool for the assessment of QoL. Scores range from 0 to 100, with higher scores indicating better QoL (Ware and Sherbourne, 1992).

The SF-12 is a subset of the SF-36 that measures the same eight domains with reduced length. It is a practical alternative for the SF-36, especially in the overall physical and mental health outcomes, and yields two different global scores: Physical Component Summary (PCS) and Mental Component Summary (MCS) (Gandek *et al.*, 1998).

The QoL assessment instrument WHOQOL-bref is a brief questionnaire comprising 26 items, including two items for overall QoL and general health, and another 24 items categorized in four broad domains (physical, psychological, social, and environmental health). Scores for each item range from one to five, with the highest score indicating the best QoL for the corresponding item (The World Health Organization quality of life assessment (WHOQOL): Position paper from the World Health Organization, 1995).

The EQ-5D is generic instrument that includes five dimensions: mobility, self-care, daily activities, pain, and emotional well-being (depression or anxiety). Each item is scored based on a three-point scale, and the EQ-5D score is calculated by their sum, resulting in scores ranging from 0 (best possible status) to 10 (worst possible status) (Touboul *et al.*, 2013).

QoL in patients with endometriosis may also be assessed using disease-specific questionnaires, such as the EHP-30. This is a self-reported questionnaire consisting of a core instrument made up of 30 items covering pain, control and powerlessness, emotional well-being, social support, and self-image; and a modular part with a total of 23 items distributed in six areas: work, relationship with children, sexual relationship, feelings about medical profession, feelings about treatment, and feelings about infertility. Each scale is standardized on a score ranging from 0

to 100, where the lowest score represents the best health status. The EHP-5, on the other hand, is a shorter version consisting of five items in the core and six items in the modular questionnaire, which has been proven as reliable in settings where a less time-consuming instrument is required (Aubry *et al.*, 2017).

The perceived stress questionnaire (PSQ) specifically measures stress in clinical psychosomatic research, and consists of 30 questions that correlate with symptomatic complaints and health outcomes (Levenstein *et al.*, 1993). In turn, the perceived stress scale (PSS) is a self-reported survey with 14 items, used to measure the characteristics of feelings and thoughts related to one's perception of stress (Cohen *et al.*, 1983).

The symptoms associated with endometriosis are known to exert substantial burden on the lives of women with endometriosis and their families. A systematic review of 20 health-related quality-of-life (HRQOL) studies showed that endometriosis was associated with pain and significant impairment of psychological and social functioning (Jia *et al.*, 2012). Diagnostic delay is significantly associated with reduced HRQOL, even after adjustment for number of symptoms. Each woman with endometriosis loses on average 11 hours of work per week, mainly owing to reduced effectiveness while working rather than absence from work, measured using the Work Productivity and Activity Index (WPAI), which is a tool to assess the effect of symptoms on effectiveness at and absence from work and ability to carry out other non-work activities (Fourquet *et al.*, 2011). As a consequence, endometriosis has a substantial socio-economic effect on the individual and on society in general. The average annual costs and HRQOL per woman with endometriosis-associated symptoms were calculated as €9,579, with two thirds of this sum solely owed to the loss of productivity (Simoens *et al.*, 2012).

Furthermore, many women with endometriosis are likely to have significant concerns about their fertility. These concerns could be expected to increase both anxiety and pain catastrophising (an exaggerated negative response in anticipation of pain) thereby amplifying the pain experience. Similarly, the association of endometriosis with dyspareunia could be expected to increase psychological distress in these women. Furthermore, if this symptom significantly impacts on their relationship, both self-esteem and social support may be reduced, potentially further worsening their pain experience (Culley *et al.*, 2013). It is plausible that these central changes contribute to the well-established disparity between the extent of disease observed at laparoscopy and the pain experienced and to the persistence of pain despite adequate surgical treatment.

Thus, multifactorial impacts of endometriosis span the life course of affected women, from the point of symptom onset onward through decades of a woman's life. Endometriosis (and its associated symptoms) has been shown to hamper educational attainment, hinder work productivity,

alter career choices and success, impair social life and activities, affect family choices, induce strain in personal relationships, negatively influence mental and emotional health, and adversely affect QoL. These multiple and pervasive effects are anticipated to materially alter the life-course trajectory of women with endometriosis (Missmer *et al.*, 2021).

4.4.1 Surgical treatment

4.4.1.1 Research study 3:

Surgical treatment of endometriosis: prognostic factors for better quality of life

Aim of the study

Endometriosis is a chronic benign disease that affects women in the reproductive age (Greene *et al.*, 2016; Zondervan, Becker, Koga, Missmer, Taylor, and Vigano, 2018), with a negative impact on quality of life (QoL) due to painful symptoms, infertility and high levels of perceived stress (Lazzeri, Orlandini, *et al.*, 2015; Nnoaham *et al.*, 2011b; Petrelluzzi *et al.*, 2008). Medical therapy and surgical interventions are available for the management of endometriosis (Bedaiwy *et al.*, 2017; Rafique and Decherney, 2017; Vercellini *et al.*, 2014). Laparoscopy is prescribed in patients with painful symptoms and subfertility in order to remove endometriotic lesions and adhesions and restore the pelvic anatomy (Flyckt *et al.*, 2017; Macer and Taylor, 2012; Tanbo and Fedorcsak, 2017). Surgery is required also for patients with contraindications or poor response to medical therapies, for acute pelvic pain events and to make differential diagnosis with malignant adnexal mass (Singh and Suen, 2017). However, as a chronic and heterogeneous disease, endometriosis is seldom treated just once with a definitive therapeutic approach (Sibiude *et al.*, 2014) and requires a life-long management plan (Kuznetsov *et al.*, 2017).

Recurrence of symptoms or lesions is highly concerning in patients who have undergone surgery for endometriosis (Bozdag, 2015; Koga *et al.*, 2015). The surgical treatment is planned to remove the disease as completely as possible, but residual foci may be left behind due to incomplete diagnosis, technical difficulties and conservative surgical interventions often performed in young patients who wish to conceive (Alimi *et al.*, 2018). In addition, new lesions may arise and reactivate the disease after a temporary therapeutic achievement (Guo, 2009; Singh and Suen, 2017). The best indicator of a successful surgical treatment of endometriosis is a complete and durable symptom relief, with the achievement of good physical and mental health (Arcoverde *et al.*, 2019). This outcome depends on the completeness of the surgical treatment, but other prognostic factors may also be relevant in order to offer the best information to patients undergoing surgery.

The aim of the present study was to investigate whether there is any association between specific features of women with surgically treated endometriosis and impaired post-surgery QoL, measured by the SF-12 questionnaire.

Materials and Methods

Study Design

A prospective survey was performed including 153 premenopausal women at mean age 36 ± 3 years old, and regular BMI (22 ± 2,0 Kg/m^2) with surgical and histological diagnosis of endometriosis. Data were collected after routine outpatient care at the Gynecologic Clinic of the University of Siena/ Italy, by using our Endometriosis Archiving Software (ENEAS) database (Centini *et al.*, 2017), and validated through review of medical records of all participants. The study was approved by the local Institutional Review Board and all participants provided written informed consent to be included in the series.

The database contained all information about: a) demographic and clinical characteristics (age, BMI, age at menarche, age at diagnosis of endometriosis, current pelvic pain severity); b) surgical treatments (age at each operation, localization of endometriotic lesions at first surgery, surgeon expertise and type of surgical technique at first operation, post-operative medical therapy, total number of surgeries and time since first surgery); c) recurrence of lesions (identified by imaging or surgical visualization and/or histology) or symptoms (dysmenorrhea, dyspareunia, non-cyclic pelvic pain). The current pelvic pain severity was assessed by Visual Analogue Scale (VAS) and defined as no pain (VAS ≤4), mild pain (VAS 5-6), moderate pain (VAS 6-7), severe pain (VAS ≥8). The localization of endometriotic lesions was classified according to the three phenotypes: OMA, SUP, DIE and mixed phenotypes.

Study Protocol

All participants answered to a validated tool for health-related QoL, the Short Form 12 (SF-12), comprising both mental and physical component scales. This instrument contains 12 items selected from the SF-36, including at least one item from each of the SF-36 scales. SF-12 scores correlate strictly with those of SF-36, but SF-12 has the advantages of being shorter and having country-specific scoring systems adjusted for each population (Gandek *et al.*, 1998).

As in the Italian general population the median physical and mental scores of SF-12 are reported to be 51.2 and 47.8, respectively , we have used these cut-offs for identifying two groups among the participants. Group A was composed by patients who had SF-12 scores above the median in both physical and mental scales, and they were, therefore, considered the patients with a satisfactory QoL. Group B comprised the patients who presented at least one score (physical and/or mental) below the medians, and it consisted of the cluster with an impaired QoL. By identifying these two groups, we aimed to compare if the patients' characteristics (i.e, demographic and clinical features, information about the surgery/s and endometriosis recurrence) would differ according to

the health-related QoL, as measured by the SF-12 questionnaire.

Data evaluation and statistical analysis

Statistical analysis was performed using IBM SPSS Statistics software, version 22 (IBM Corporation, Armonk, NY, USA). Statistically significant differences between groups were determined using Mann-Whitney U test and Chi-square test or Fisher's exact test. A p value <0.05 was considered statistically significant.

We performed a stepwise backward logistic regression to assess potential clinical characteristics independently associated with SF-12 scores. Data are reported as adjusted odds ratios (OR) with 95 percent confidence intervals (95% CI).

The sample size was estimated to detect differences of at least 0.5 standard deviations in quantitative variables or 20% in the frequency of categorical variables between groups with 80% statistical power and 95% confidence level.

Results

In the study sample, the median physical and mental scores of SF-12 were 44 and 35, respectively, and they did significantly differ to the Italian population normal values (p=0.0001). The distribution of physical and mental SF-12 scores in the study participants is shown in Figure 1, which displays also the mean value of the representative sample of the general Italian population [22]. According to these cut-offs, Group A (n=42) and Group B (n=111) were compared.

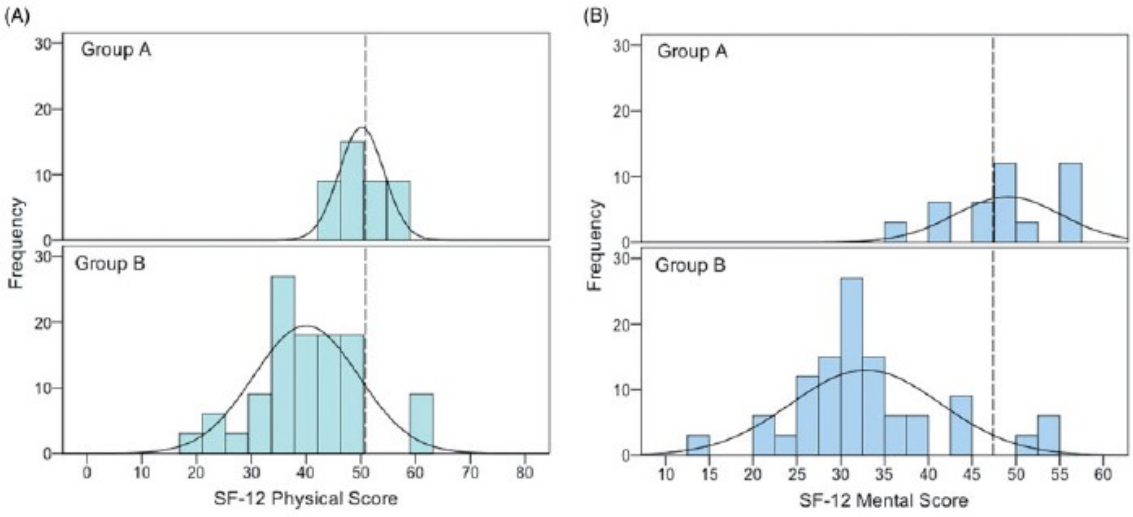


Figure 1. Frequency distribution of SF-12 physical (A) and mental (B) scores of women with endometriosis in the present study. The dotted lines indicate the mean value of a representative sample of the general Italian population.

The two groups did not differ in terms of actual age or BMI. However, Group A has been diagnosed and operated for endometriosis for the first time at an older age (30 vs. 26 years, $p<0.001$) and the surgery has been done more recently (82 vs. 115 months, $p<0.01$) than in Group B. More patients of Group A had undergone a single surgical intervention (64% vs. 46%, $p<0.05$) and Group A was less affected by symptom or lesion recurrence than Group B (Table 1). Current pelvic pain was less intense in Group A compared to that reported by Group B (Chi-square for linear trend = 10.9, $p = 0.001$, Figure 2).

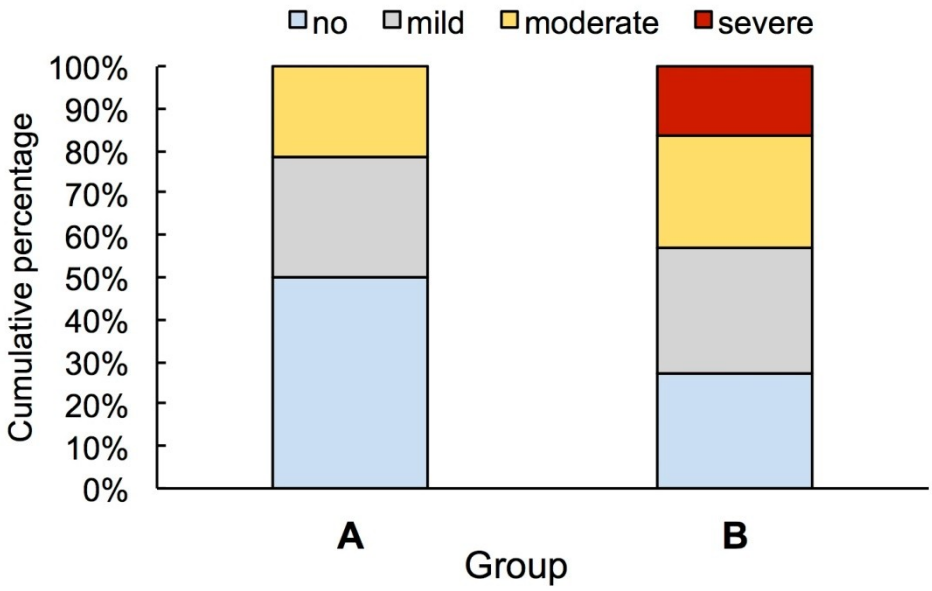


Figure 2. Proportion of women currently experiencing several degrees of pelvic pain in the two study groups.

As shown in Table 1, Groups A and B differed regarding the endometriosis lesions' localization. The association of the three phenotypes was more frequently observed in Group B ($p=0.0001$). Similarly, coexistent superficial and ovarian phenotypes were more commonly found in Group B, whereas the presence of DIE lesions alone were more often detected in Group A. There were no differences in terms of the surgeon who performed the operation (gynecologist, general surgeon or multidisciplinary team) and the technique used (laparoscopy, laparotomy or both).

Regarding the post-operatory hormonal therapy, Group A underwent more frequently to GnRH agonist or progestins treatment, whereas in Group B multiple hormonal therapies were administered ($p=0.001$). Multivariable logistic regression indicated that having the first endometriosis surgery at a later age was an independent predictor of better health status (adjusted odds ratio 1.146 per year, 95% confidence interval 1.058 to 1.242, $p=0.001$) after accounting for the potential confounding effects of reoperation and non-cyclic pelvic pain (Table 2).

Table 1. Clinical characteristics of the study groups.

	Group A (n = 42)	Group B (n = 111)	P value
Age (years)	36.6 ± 4.4	36.1 ± 7.0	0.230
BMI (Kg/m^2)	21.9 ± 2.6	22.1 ± 3.4	0.868
Age at menarche (years)	12.1 ± 0.8	12.5 ± 1.4	0.052
Age at first surgery (years)	30.4 ± 5.5	26.5 ± 5.0	0.000
Lesions at first surgery			
SUP only	6 (14%)	6 (5%)	0.000
OMA only	15 (36%)	51 (46%)	
DIE only	6 (14%)	0 (0%)	
SUP + OMA	3 (7%)	18 (16%)	
SUP + DIE	3 (7%)	3 (3%)	
OMA + DIE	6 (14%)	12 (11%)	
SUP + OMA + DIE	3 (7%)	21 (19%)	
Surgeon			
Gynecologist	33 (79%)	90 (81%)	0.481
General surgeon	3 (7%)	3 (3%)	
Multidisciplinary team	6 (14%)	18 (16%)	
Technique			
Laparoscopy	33 (79%)	93 (84%)	0.477
Laparotomy	6 (14%)	15 (13%)	
Both	3 (7%)	3 (3%)	
Post-operative medical therapy			
None	9 (21%)	24 (22%)	0.001
GnRH agonist	13 (36%)	21 (19%)	
Progestin	15 (36%)	30 (27%)	
Combined hormonal contraceptive	3 (7%)	12 (11%)	
Multiple hormonal therapy	0 (0%)	24 (22%)	
Time since first surgery (months)	82 ± 51	115 ± 69	0.003
No. of surgeries	1.6 ± 0.9	2.0 ± 1.2	0.047
Two or more surgeries (n, %)	15 (36%)	60 (54%)	0.042
Recurrence of pain (n, %)			
Dysmenorrhea	21 (50%)	75 (68%)	0.047
Dyspareunia	9 (21%)	27 (24%)	0.704
Non-cyclic pelvic pain	12 (29%)	57 (51%)	0.010
Recurrence of lesion (n, %)	21 (50%)	75 (68%)	0.047

Group A: women with SF-12 scores above the median in both physical and mental scales.
Group B: women with SF-12 scores below the median in either physical or mental scale.
SUP = superficial peritoneal endometriosis; OMA = ovarian endometrioma; DIE = deep infiltrating endometriosis

Table 2. Multivariable logistic regression for variables possibly associated with good health status, defined as SF-12 above the median (Group A).

Variable	Adjusted OR	95% CI	P value
Age at first surgery (per year)	1.146	1.058 – 1.242	0.001
Non-cyclic pelvic pain	0.435	0.271 – 0.698	0.001
Two or more surgeries	0.843	0.313 – 2.271	0.735

Discussion

The present study showed that patients with endometriosis and impaired QoL had first surgery at a younger age, more symptoms recurrence and more reoperations. Therefore, having the first endometriosis surgery at a later age was an independent predictor of better health condition.

A timely first surgery plays a pivotal role in a life-long treatment of patients affected by endometriosis and a not well-performed intervention often makes the subsequent one harder. Cheong *et al* showed that age is the main factor that influences the likelihood of repeated surgery, which could be linked to increased aggressiveness of the disease when it occurs in young women or to a surgeon's tendency to be less radical in the approach of younger patients (Cheong *et al.*, 2008). The first surgery for endometriosis should be based on pain and desire for pregnancy not responsive to medical treatment or ART (Singh and Suen, 2017). Asymptomatic young patients who do not wish a pregnancy in the near future should be informed about the possibility of post-operatory recurrence and the risks and difficulties related to additional surgeries. In fact, an increased risk of disease recurrence and repeated operations was found in women having their index surgery before 30 years old (Shakiba *et al.*, 2008). In case of endometrioma, young patients must be also informed about the possible reduction of ovarian function and ovarian loss, as a result of the surgery (Berlanda *et al.*, 2010; Chiang *et al.*, 2015; Kho *et al.*, 2018; Nowak-Psiorz *et al.*, 2019; Santulli *et al.*, 2016).

Our results showed a higher prevalence of coexistent phenotypes (SUP+OMA; SUP+OMA+DIE) in women with impaired QoL. Furthermore, those who had a worse QoL have been submitted to multiple surgical interventions. As shown by Sibiude *et al*., patients with previous surgeries for endometriosis are more likely to have DIE and a higher disease stage at the time of the surgery (Sibiude *et al.*, 2014). Indeed, the number of operations increases the patient's perceived stress, suggesting an adverse effect of multiple surgeries on mental and psychological state (Lazzeri, Vannuccini, *et al.*, 2015b).

Despite the improvement of surgical techniques and the advances in preoperative evaluation of the extension of the disease (Exacoustos, Malzoni, *et al.*, 2014; Guerriero *et al.*, 2016; Nisenblat

et al., 2016), the recurrence of endometriosis and the need of repeated surgery remain frequent outcomes (Berlanda *et al.*, 2010). The reappearance of symptoms and endometriotic lesions increases over the time and varies according to the subtype of the disease and surgery-related variables (Bozdag, 2015). The recurrence rates described reach 22% at 2 years of post-intervention and 40-50% at 5 years, and the probability of a further surgical procedure is about 15-20% (Donnez and Squifflet, 2010; Saraswat *et al.*, 2018; Vercellini, Barbara, *et al.*, 2009). The present study showed that patients with symptoms and lesion recurrence have the worse SF-12 scores, reinforcing the negative relationship of endometriosis recurrence and physical and mental wellbeing (Culley *et al.*, 2013). Pain is a crucial symptom in determining the health status, in fact women with chronic pelvic pain had poorer QoL and mental health (Facchin *et al.*, 2015). Furthermore, those with severe pain showed a higher incidence of multiple psychiatric disorders (Vannuccini *et al.*, 2018).

A pivotal role after surgical treatment is played by medical treatment (Rocha *et al.*, 2012; Somigliana *et al.*, 2014, 2017; Vercellini *et al.*, 2003), in order to prevent recurrence (Tobiume *et al.*, 2016; Wu *et al.*, 2018) and to manage symptoms (Vercellini *et al.*, 2018). The use of a post-operative medical therapy has been widely addressed in literature for patients who are not seeking conception (Somigliana *et al.*, 2014). Our results showed, from one hand, that the patients with satisfactory QoL underwent more often to post-operative progestins or GnRH analogs treatment, whereas the use of multiple hormonal drugs was more common in those with low QoL scores. Long term treatment with progestins have been shown to reduce pain, improve health perception and prevent recurrence (Andres *et al.*, 2015; Belaisch, 2009; Römer, 2018; Vercellini, Bracco, *et al.*, 2016; Vercellini, Buggio, *et al.*, 2016). Similarly, GnRH agonists treatment after surgery for six months significantly decreases the recurrence rate of endometriosis (Takaesu *et al.*, 2016; Zheng *et al.*, 2016). The use of multiple therapies in Group B may reflect a more aggressive behavior of endometriosis, pharmacologic resistance and a worse clinical presentation of the disease in those who presented with low QoL.

One methodological strength of this study is the standardized documentation of clinical data on a dedicated database. In addition, the follow-up interviews were performed face-to-face, reducing the risk of recall bias. However, there are some limitations to acknowledge: patients who had undergone either single or multiple surgeries were included; all preoperative and surgical data were obtained from medical files and reports, despite the patients being interviewed prospectively. Furthermore, additional variables have not been assessed in the present study such as socioeconomic status, previous mental health, sexuality, coexisting painful conditions and lifestyle habits, that, among others, could also have influence over the health status of the patients (Lövkvist *et al.*, 2016; Pluchino *et al.*, 2016). It should be also observed that the association of a worse QoL

and an younger age at the first surgery could reflect an initial less aggressive approach because of less severe forms of the disease. In fact, Groups A and B significantly differ in terms of lesions' localization. Unfortunately, no information about the surgical ASRM classification were available, so we used the lesions' localization as a proxy for endometriosis extension, although this may not always provide a reliable picture of the severity of the disease. The endometriosis recurrence, on the other hand, could be also related to the experience and technical capabilities of the surgical team (Exacoustos *et al.*, 2017). Our results, nevertheless, did not show any differences in terms of QoL depending on the surgeon who performed the operation However, the small number of patients operated by a general surgeon or a multidisciplinary team does not allow to draw final conclusions.

In summary, our results suggest that patients with endometriosis undergoing first surgery at young age reported an increased frequency of multiple surgeries and disease recurrence, showing a poor physical and mental health status. Preoperative counseling is a key step of endometriosis management and the prognostic variables identified here may help the surgeon to better inform the patient about her odds of gaining a satisfactory health condition after pursuing this complex treatment.

4.4.2 Medical treatment

4.4.2.1 Research study 4

Long-term hormonal treatment reduces repetitive surgery for endometriosis recurrence

Aim of the study

Endometriosis is a chronic inflammatory disease with a prevalence of 10% in reproductive age that has endocrine and immunological disturbances among its pathogenetic factors (Clemenza *et al.*, 2018). The clinical management of women with endometriosis is quite complex and depends on patient's age and symptoms. Particularly, patients with endometriosis-related pain have two therapeutic options: medical or surgical treatment. For a long period of time minimally invasive surgery was recognized as the standard approach to treat endometriosis, also in order to obtain a clear histological diagnosis. The surgical approach may be conservative (removal of endometriotic lesion) or definitive (hysterectomy with or without oophorectomy) (Falcone and Flyckt-Rebecca, 2018). However, a high recurrence rate of pain symptoms is described at 2 years post-operatively (21.5%) and it is even higher at 5 years follow up (40-50%) (Guo, 2009).

The debate regards if the disease recurrences are the results of de novo lesions after surgery or the incomplete surgical treatment of endometriosis. Supporting this last hypothesis, some studies have shown that performing a complete first surgery drives to a decreased recurrence rate (Sibiude *et al.*, 2014), that the anatomical distribution of recurrent lesions is similar to that found in the first surgery (Taylor and Williams, 2010) and that the execution of the first surgery by a highly expert operator is associated to lower number of recurrences (Ceccaroni *et al.*, 2019). Anyhow, the repetitive surgery for endometriosis occurs and has been described (Ceccaroni *et al.*, 2019; Horne *et al.*, 2019); it is much more complex than first surgery because of the presence of peritoneal and visceral adhesions (Carmona *et al.*, 2009).

Since endometriosis patients face the risk of recurrence of pain and repetitive surgery, more information is required before a treatment is started in order to establish the best appropriate management (Falcone and Flyckt-Rebecca, 2018). In fact, endometriosis is now recognized as a chronic disease and evidence suggests that a modern management should postpone the first surgery (Chapron *et al.*, 2019) and prescribe a post-operative medical treatment (Dunselman *et al.*, 2014; Saridogan *et al.*, 2017; *et al.*, 2017, 2020). The present study aimed to identify the role of medical treatment both before to plan a surgery and after surgery aiming to preventing endometriosis recurrence, reducing repetitive surgery for recurrence. The impact on quality of life was also investigated.

Material and Methods

An observational cross-sectional study was conducted in reproductive age women (n=185) with endometriosis, recruited in two different hospitals (Florence and Negrar di Valpolicella, Italy) between January 2017 and February 2020. Data were collected by an extensive review of clinical records of patients in follow-up in these Endometriosis outpatient clinics. Inclusion criteria were: fertile age (25-45 years), previous surgery for endometriosis, no desire of pregnancy, nulliparity. Women wishing to become pregnant when the survey was conducted or those who had previously tried to conceive, both naturally or through ART, were excluded from the study. Included patients were divided into three groups according to the hormonal treatment received: Group A (n=34), no hormonal treatment neither before nor after the first endometriosis surgery, Group B (n=76) on hormonal treatment after the first endometriosis surgery and Group C (n=75) on hormonal treatment both before and after the first endometriosis surgery.

The used hormonal treatments were: progestins (40%), GnRH analogs (30%) or continuous oral contraceptives (30%), for a minimum of 12 months before and prolonged for 2 years or more after surgery (also changing the type of hormonal treatment). All patients have been operated in referral centres for endometriosis surgery and a histological confirmation of the disease was available for each case. Women were followed up for at least 2 years after the first endometriosis surgery. OMA, DIE and SUP were the phenotypes identified (Table 1). During the follow-up visit, patients were interviewed through: 1) a structured questionnaire containing all clinical information regarding the history of the disease (in particular, the data regarding the first surgery); 2) the assessment of current situation by administering a validated tool for health-related quality of life (QoL), the Short Form 12 (SF-12), comprising both mental and physical component scales. This instrument contains 12 items selected from the SF-36, including at least one item from each of the SF-36 scales. SF-12 scores correlate strictly with those of SF-36, but SF-12 has the advantages of being shorter and having country-specific scoring systems adjusted for each population (Gandek *et al.*, 1998).

In case of repetitive endometriosis surgery, the indications for re-operations were the presence of painful symptoms resistant to medical treatment or the imaging evidence of endometriosis lesions recurrence associated to pain.

The study was approved by the local Institutional Review Board (protocol n.14558 approved on 30.05.2019) and all participants provided written informed consent to be included in the series.

Statistical analysis was performed using IBM SPSS Statistics software, version 22 (IBM Corporation, Armonk, NY, USA). Statistically significant differences between groups were determined using Student's *t*-test for continuous variables and Chi-square test or Fisher's exact test

for categorical variables. Survival analysis was performed by the Kaplan-Meier method setting the time to reoperation as time variable and censoring cases at the time of the second surgery or at the date of follow-up. The curves were compared by the Log Rank test and then adjusted by stepwise forward Cox regression to any potential confounding variable. A p value <0.05 was considered statistically significant.

Results

Age at endometriosis diagnosis was significantly higher in Group A compared with Group B and Group C (p=0.009; Table 1).

Pointing our attention to the first endometriosis surgery, the age of the patients was similar in the 3 groups of patients (Table 1) and the gynecologists performed more frequently the first endometriosis surgery than general surgeons or multidisciplinary teams (100%, 73.7% and 80% in groups A, B and C, respectively, p=0.001), preferring laparoscopy in most cases, especially in groups A and C (p=0.002). Pelvic pain was the main symptom leading to the first surgery in groups B and C (p=0.004). Among the 3 phenotypes, the OMA was the most frequent endometriosis phenotype at first endometriosis surgery in the three groups (p=0.010) (Table 1).

When the rate of repetitive endometriosis surgery for recurrence was evaluated, the proportion of women that underwent reoperation was lower in those patients treated with hormones before and after (Group C, 45.3%) than in those treated only after the first endometriosis surgery (Group B, 57.9%) or in those without any treatment (Group A, 70.6%, chi-square 6.394, p= 0.011, Table 2).

The survival analysis showed that the probability of reoperation over time differed significantly between the treatment groups (Log Rank test, p = 0.008) even after adjustment to the main symptom and to the type of surgery (hazard ratio 2.57, 95% CI 1.47 - 4.49). This difference between the three groups persisted over the years (Figure 1). The median time to reoperation estimated by the Kaplan-Meier analysis was 3 years, 5 years and 9 years in groups A, B and C, respectively (p = 0.008, Table 2).

Considering the current clinical situation, patients of Group C were characterized by a lower rate of dysmenorrhea compared with patients of Group B (59.5% versus 81.9%) (p=0.006). The rates of dyspareunia, urinary pain, dyschezia and heavy menstrual bleeding were similar between the three groups (data not shown).

Women with repetitive surgery for endometriosis recurrence showed a lower SF-12 physical (p=0.004) and mental (p=0.012) scores than those who received a single surgery, independently from the treatment (Figure 2).

Table 1: First surgery characteristics.

Hormonal treatment before/after the first endometriosis surgery	**None (n = 34)**	**Only After (n = 76)**	**Before + After (n = 75)**	**p**
Age at 1st diagnosis (yr.)	30.0 ± 4.9	27.0 ± 5.2	26.6 ± 6.3*	0.009
Age at 1st surgery for endometriosis (yr.)	29.7 ± 5.8	27.2 ± 5.3	27.8 ± 5.9	0.107
Pain as main symptom	12 (35.3%)	46 (60.5%)	45 (60.0%)	0.004
Who performed 1st surgery				
Gynaecologist	34 (100.0%)	56 (73.7%)	60 (80.0%)	0.001
General surgeon	0 (0.0%)	9 (11.8%)	0 (0.0%)	
Multidisciplinary team	0 (0.0%)	11 (14.5%)	15 (20.0%)	
Type of 1st surgery				
Laparoscopy	31 (91.2%)	56 (74.7%)	68 (90.7%)	0.002
Laparotomy	0 (0.0%)	16 (21.3%)	7 (9.3%)	
Laparoscopy converted in laparotomy	3 (8.8%)	3 (4.0%)	0 (0.0%)	
Endometriosis phenotype at 1st surgery				
OMA	19 (55.9%)	45 (59.2%)	45 (60%)	0.010
DIE	0 (0%)	12 (15.8%)	3 (4%)	
SUP	4 (11.8%)	7 (9.2%)	3 (4%)	
OMA+DIE	11 (32.4%)	12 (15.8%)	24 (32%)	

Continuous data were summarized as means ± SD and compared by one-way ANOVA followed by Student-Newman-Keuls test for multiple comparisons; *p<0.05 Before + After vs. "None".
Categorical variables were compared by Chi-square test.

Table 2: Follow-up and outcomes after the first surgery.

Hormonal treatment before/after the first endometriosis surgery	**None (n = 34)**	**Only After (n = 76)**	**Before + After (n = 75)**	**p**
Length of follow-up (years)*	7.9 ± 5.9	8.6 ± 5.9	8.5 ± 4.7	0.829
Length of post-operative hormonal therapy				
1-12 months	–	52/70 (74.3%)	46/72 (63.9%)	0.300
13-24 months	–	3/70 (4.3%)	7/72 (9.7%)	
>24 months	–	15/70 (21.4%)	19/72 (26.4%)	
Rate of symptom recurrence (%)				
During hormonal therapy	–	41/69 (59.4%)	38/72 (52.8%)	0.432
After therapy suspension	–	16/69 (23.2%)	15/72 (20.8%)	
Time from therapy suspension to symptom recurrence[#]				
1-12 months	–	14/16 (87.5%)	14/15 (93.3%)	1.000
>12 months	–	2/16 (12.5%)	1/15 (6.7%)	
Rate of reoperation (%)	24 (70.6%)	44 (57.9%)	34 (45.3%)	0.011
Interval between 1st and 2nd surgery (years)[§]	3.0 (1.4-4.5)	5.0 (3.8-6.2)	9.0 (6.9-11.1)	0.008

*Means ± standard deviations, compared by one-way ANOVA.
Categorical variables were compared by Chi-square test.
[#]Considering only the women who had a recurrence after discontinuing the post-operative hormonal treatment.
[§]Median (95% confidence interval) according to Kaplan-Meier's survival analysis.

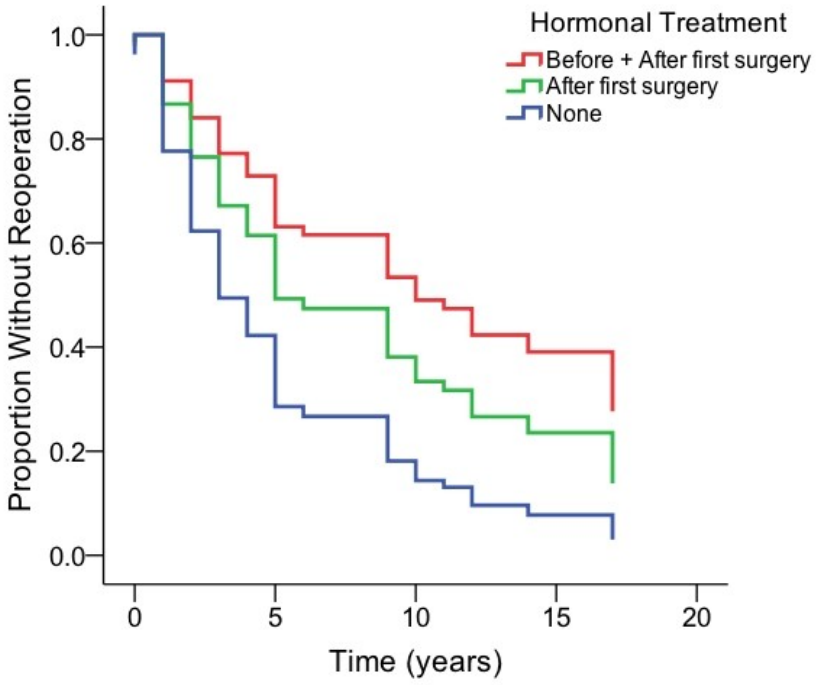


Figure 1: Survival plots (Cox regression) showing the time to reoperation in women who received no medical treatment, medical treatment after the first endometriosis surgery or medical treatment before + after the first endometriosis surgery. Log Rank test, p = 0.008 after adjustment to the main symptom and to the type of surgery hazard ratio = 2.57, 95% CI 1.47 - 4.49.

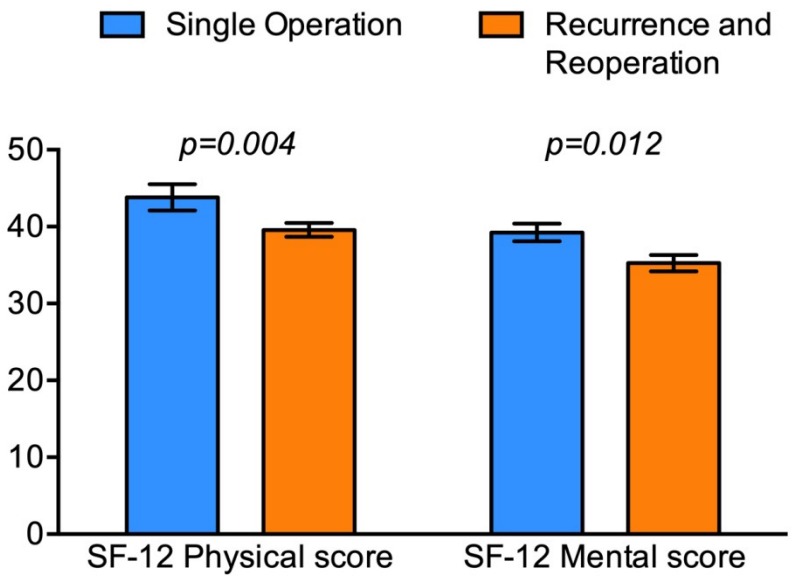


Figure 2: SF-12 Physical and mental score in single operation (n=83) patients and those with repeated surgery for endometriosis recurrence (n=102). Data are means ± standard errors. The p values refer to Student's *t*-test.

Discussion

The present study showed a reduced incidence of endometriosis recurrence leading to reoperation in those patients treated with hormonal treatment both before and after the first endometriosis surgery and the same group of patients is characterized by a lower rate of current dysmenorrhea, compared with women who received hormonal treatment only after surgery. These data support a possible role of the hormonal treatment for a certain period of time (independently from progestins, GnRH analogs or continuous oral contraceptives) in postponing the first surgery, in preventing repetitive surgery for recurrence and in controlling dysmenorrhea. In fact, in order to effectively reduce endometriosis recurrence, the administration of hormonal treatment should be long-term, ideally until patient desire to conceive or until menopause, rather than just for a few months (Chapron *et al.*, 2019). These data are in agreement with previous observations showing that the administration of a post-operative treatment with progestins, GnRH analogs or continuous oral contraceptives is effective in preventing recurrences of endometriosis (Abou-Setta *et al.*, 2013; Ceccaroni *et al.*, 2019; Cho *et al.*, 2014; Koshiba *et al.*, 2018; Seo *et al.*, 2017).

The surgical management of endometriosis still remains an important therapeutic option. It is specifically indicated in case of failure, intolerance and contraindication of medical treatment, for infertility treatment in younger women wishing to conceive naturally and in cases of DIE with bowel occlusion or ureteral DIE with ureterohydronephrosis (Chapron *et al.*, 2019; Falcone and Flyckt-Rebecca, 2018). However, since endometriosis surgery is characterized by potential

operative complications and multiple recurrences (Vercellini, Somigliana, *et al.*, 2009) and since it does not treat the pathogenetic mecahnisms of the disease (Vercellini, Crosignani, *et al.*, 2009) and may have a negative effect on ovarian reserve (Raffi *et al.*, 2012), the choice of surgical treatment should be adequately evaluated and personalized. The high post-operative recurrence rate may explain the use of repetitive surgery for endometriosis in about 50% of the patients (Cheong *et al.*, 2008; Vannuccini *et al.*, 2019). A young age at the first endometriosis surgery (Sibiude *et al.*, 2014; Vannuccini *et al.*, 2019), and in particular <35 years (Selcuk *et al.*, 2016) is considered a risk factor for recurrence. Our present data support a prolonged preoperative hormonal treatment with the aim of postponing the first surgery. Other risk factors are associated with endometriosis recurrence and should be taken into account before establishing the best therapeutic management: family history (Campo *et al.*, 2014), body mass index ≥23 kg/m^2(Nirgianakis *et al.*, 2014), the presence of large endometrioma and high serum level of CA-125 (Cho *et al.*, 2014; Küçükbaş *et al.*, 2018) and an advanced stage of the disease (Tobiume *et al.*, 2016). In this context, as previously demonstrated, a more radical surgery is associated with a lower rate of recurrences (Busacca *et al.*, 2006; Ceccaroni *et al.*, 2019).

Endometriotic patients have a low quality of life and the present study confirms that patients experiencing repetitive surgery for endometriosis recurrences are characterized by lower SF-12 physical and mental scores than patients receiving a single surgical treatment. Severe pain may cause a worse mental and physical QoL, as well as the perceived stress (Lazzeri, Vannuccini, *et al.*, 2015b; Vannuccini *et al.*, 2018) and stress may reduce immune response in multi-operated patients, explaining the development of disease recurrences or comorbities (*Reis et al., 2020*). In fact, a combined effect of high chronic pain and low global quality of life can lead to the so-called sickness response, associated with a vicious cycle caused by the underlying neuroendocrine–immune imbalance. Therefore, the stress condition may be a risk factor for endometriosis recurrence and repetitive surgery (Reis, Coutinho, Vannuccini, Luisi, *et al.*, 2020).

These data support the modern concept that the gold standard for an appropriate endometriosis management is the individualized approach and surgery should be considered depending on clinical situation and patient's symptoms. In fact, clinical and ultrasonographic diagnosis of endometriosis, above all in young women, should not drive to an immediate surgical treatment (Chapron *et al.*, 2019). In the diagnostic process, the presence of gynaecological comorbidities, such as uterine disorders (adenomyosis and uterine fibroids) should be also accurately evaluated because it may influence the consequent treatment choice (Capezzuoli *et al.*, 2020).

The present study confirmed that hormonal treatments represent a valid cornerstone of endometriosis management and it may be useful as alternative to surgery, but also before surgery for a better planning and after surgery in order to reduce the risk of recurrence. In this context, the counselling of patients by clinicians may be very helpful to choose the correct and individualized endometriosis treatment.

References

Abou-Setta AM, Houston B, Al-Inany HG, Farquhar C. Levonorgestrel-releasing intrauterine device (LNG-IUD) for symptomatic endometriosis following surgery. *Cochrane Database Syst Rev* 2013.

Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, Singh SS, Taylor HS. Clinical diagnosis of endometriosis: a call to action. *Am J Obstet Gynecol* 2019.

van Aken M, Oosterman J, van Rijn T, Ferdek M, Ruigt G, Kozicz T, Braat D, Peeters A, Nap A. Hair cortisol and the relationship with chronic pain and quality of life in endometriosis patients. *Psychoneuroendocrinology* 2018.

Alimi Y, Iwanaga J, Oskouian RJ, Loukas M, Tubbs RS. The clinical anatomy of dyspareunia: A review. *Clin Anat* 2018.

Alio L, Angioni S, Arena S, Bartiromo L, Bergamini V, Berlanda N, Bonin C, Busacca M, Candiani M, Centini G, *et al.* When more is not better: 10 'don'ts' in endometriosis management. An ETIC* position statement. *Hum Reprod Open* 2019.

AlKudmani B, Gat I, Buell D, Salman J, Zohni K, Librach C, Sharma P. In Vitro Fertilization Success Rates after Surgically Treated Endometriosis and Effect of Time Interval between Surgery and In Vitro Fertilization. *J Minim Invasive Gynecol* 2018.

Álvarez-Salvago F, Lara-Ramos A, Cantarero-Villanueva I, Mazheika M, Mundo-López A, Galiano-Castillo N, Fernández-Lao C, Arroyo-Morales M, Ocón-Hernández O, Artacho-Cordón F. Chronic fatigue, physical impairments and quality of life in women with endometriosis: A case-control study. *Int J Environ Res Public Health* 2020.

Andres M de P, Lopes LA, Baracat EC, Podgaec S. Dienogest in the treatment of endometriosis: systematic review. *Arch Gynecol Obstet* 2015.

Andres MP, Borrelli GM, Ribeiro J, Baracat EC, Abrão MS, Kho RM. Transvaginal Ultrasound for the Diagnosis of Adenomyosis: Systematic Review and Meta-Analysis. *J Minim Invasive Gynecol* 2018.

Appleyard CB, Cruz ML, Hernandez S, Thompson KJ, Bayona M, Flores I. Stress management affects outcomes in the pathophysiology of an endometriosis model. *Reprod Sci* 2015;**22**.

Arcoverde FVL, Andres M de P, Borrelli GM, Barbosa P de A, Abrão MS, Kho RM. Surgery for Endometriosis Improves Major Domains of Quality of Life: A Systematic Review and Meta-Analysis. *J Minim Invasive Gynecol* 2019.

As-Sanie S, Black R, Giudice LC, Gray Valbrun T, Gupta J, Jones B, Laufer MR, Milspaw AT, Missmer SA, Norman A, *et al.* Assessing research gaps and unmet needs in endometriosis. *Am J Obstet Gynecol* 2019.

As-Sanie S, Harris RE, Napadow V, Kim J, Neshewat G, Kairys A, Williams D, Clauw DJ, Schmidt-Wilcke T. Changes in regional gray matter volume in women with chronic pelvic pain: A voxel-based morphometry study. *Pain* 2012.

As-Sanie S, Kim J, Schmidt-Wilcke T, Sundgren PC, Clauw DJ, Napadow V, Harris RE. Functional Connectivity Is Associated with Altered Brain Chemistry in Women with Endometriosis-Associated

Chronic Pelvic Pain. In: *Journal of Pain*. 2016.

Aubry G, Panel P, Thiollier G, Huchon C, Fauconnier A. Measuring health-related quality of life in women with endometriosis: Comparing the clinimetric properties of the Endometriosis Health Profile-5 (EHP-5) and the EuroQol-5D (EQ-5D). *Hum Reprod* 2017.

Ballard K, Lane H, Hudelist G, Banerjee S, Wright J. Can specific pain symptoms help in the diagnosis of endometriosis? A cohort study of women with chronic pelvic pain. *Fertil Steril* 2010.

Barcena de Arellano ML, Arnold J, Lang H, Vercellino GF, Chiantera V, Schneider A, Mechsner S. Evidence of neurotrophic events due to peritoneal endometriotic lesions. *Cytokine* 2013.

Barrier BF, Malinowski MJ, Dick EJ, Hubbard GB, Bates GW. Adenomyosis in the baboon is associated with primary infertility. *Fertil Steril* 2004;**82**:1091–1094.

Bazot M, Daraï E. Diagnosis of deep endometriosis: clinical examination, ultrasonography, magnetic resonance imaging, and other techniques. *Fertil Steril* 2017.

Bazot M, Lafont C, Rouzier R, Roseau G, Thomassin-Naggara I, Daraï E. Diagnostic accuracy of physical examination, transvaginal sonography, rectal endoscopic sonography, and magnetic resonance imaging to diagnose deep infiltrating endometriosis. *Fertil Steril* 2009.

Bedaiwy MA, Allaire C, Yong P, Alfaraj S. Medical Management of Endometriosis in Patients with Chronic Pelvic Pain. *Semin Reprod Med* 2017.

Belaisch J. Progestins and medical treatment of endometriosis - Physiology, history and society. In: *Gynecological Endocrinology*. 2009.

Benagiano G, Brosens I, Habiba M. Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. *Hum Reprod Update* 2014;**20**:386–402.

Berlanda N, Vercellini P, Fedele L. The outcomes of repeat surgery for recurrent symptomatic endometriosis. *Curr Opin Obstet Gynecol* 2010.

Bernstein CN. The brain-gut axis and stress in inflammatory bowel disease. *Gastroenterol Clin N Am* 2017;**46**.

Bilibio JP, Souza CA, Rodini GP, Andreoli CG, Genro VK, de Conto E, Cunha-Filho JS. Serum prolactin and CA-125 levels as biomarkers of peritoneal endometriosis. *Gynecol Obs Investig* 2014;**78**.

Blackburn-Munro G, Blackburn-Munro R. Pain in the brain: Are hormones to blame? *Trends Endocrinol Metab* 2003.

Bomholt SF, Harbuz MS, Blackburn-Munro G, Blackburn-Munro RE. Involvement and Role of the Hypothalamo-pituitary-adrenal (HPA) Stress Axis in Animal Models of Chronic Pain and Inflammation. *Stress* 2004.

Borghese B, Sibiude J, Santulli P, Pillet MCL, Marcellin L, Brosens I, Chapron C. Low birth weight is strongly associated with the risk of deep infiltrating endometriosis: Results of a 743 case-control study. *PLoS One* 2015;**10**.

Van Den Bosch T, Dueholm M, Leone FPG, Valentin L, Rasmussen CK, Votino A, Van Schoubroeck D, Landolfo C, Installé AJF, Guerriero S, *et al.* Terms, definitions and measurements to describe

sonographic features of myometrium and uterine masses: A consensus opinion from the Morphological Uterus Sonographic Assessment (MUSA) group. *Ultrasound Obstet Gynecol* 2015;**46**:284–298.

Bougie O, Healey J, Singh SS. Behind the times: revisiting endometriosis and race. *Am J Obstet Gynecol* 2019.

Bougie O, Yap MI, Sikora L, Flaxman T, Singh S. Influence of race/ethnicity on prevalence and presentation of endometriosis: a systematic review and meta-analysis. *BJOG An Int J Obstet Gynaecol* 2019.

Bozdag G. Recurrence of endometriosis: Risk factors, mechanisms and biomarkers. *Women's Heal* 2015.

Brawn J, Morotti M, Zondervan KT, Becker CM, Vincent K. Central changes associated with chronic pelvic pain and endometriosis. *Hum Reprod Update* 2014.

Buck Louis GM, Hediger ML, Peterson CM, Croughan M, Sundaram R, Stanford J, Chen Z, Fujimoto VY, Varner MW, Trumble A, *et al.* Incidence of endometriosis by study population and diagnostic method: The ENDO study. *Fertil Steril* 2011.

Bungum HF, Vestergaard C, Knudsen UB. Endometriosis and type 1 allergies/immediate type hypersensitivity: a systematic review. *Eur J Obstet Gynecol Reprod Biol* 2014;**179**:209–215.

Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. *Fertil Steril* 2012;**98**:511–519.

Busacca M, Chiaffarino F, Candiani M, Vignali M, Bertulessi C, Oggioni G, Parazzini F. Determinants of long-term clinically detected recurrence rates of deep, ovarian, and pelvic endometriosis. *Am J Obstet Gynecol* 2006.

Campbell CM, Edwards RR. Ethnic differences in pain and pain management. *Pain Manag* 2012.

Campo S, Campo V, Gambadauro P. Is a positive family history of endometriosis a risk factor for endometrioma recurrence after laparoscopic surgery? *Reprod Sci* 2014.

Capezzuoli T, Vannuccini S, Fantappiè G, Orlandi G, Rizzello F, Coccia ME, Petraglia F. Ultrasound findings in infertile women with endometriosis: evidence of concomitant uterine disorders. *Gynecol Endocrinol* 2020.

Carmona F, Martínez-Zamora A, González X, Ginés A, Buñesch L, Balasch J. Does the learning curve of conservative laparoscopic surgery in women with rectovaginal endometriosis impair the recurrence rate? *Fertil Steril* 2009.

Carrarelli P, Luddi A, Funghi L, Arcuri F, Batteux F, Dela Cruz C, Tosti C, Reis FM, Chapron C, Petraglia F. Urocortin and corticotrophin-releasing hormone receptor type 2 mRNA are highly expressed in deep infiltrating endometriotic lesions. *Reprod Biomed Online* 2016;**33**:476–483.

Caserta D, Mallozzi M, Pulcinelli FM, Mossa B, Moscarini M. Endometriosis allergic or autoimmune disease: pathogenetic aspects--a case control study. *Clin Exp Obs Gynecol* 2016;**43**.

Ceccaroni M, Bounous VE, Clarizia R, Mautone D, Mabrouk M. Recurrent endometriosis: a battle against an unknown enemy. *Eur J Contracept Reprod Heal Care* 2019.

Centini G, Zannoni L, Lazzeri L, Buiarelli P, Limatola G, Petraglia F, Seracchioli R, Zupi E. Enhanced Endometriosis Archiving Software (ENEAS): An Application for Storing, Retrieving, Comparing, and Sharing Data of Patients Affected by Endometriosis Integrated in the Daily Practice. *J Minim Invasive*

Gynecol 2017.

Chamié LP, Ribeiro DMFR, Tiferes DA, De Macedo Neto AC, Serafini PC. Atypical sites of deeply infiltrative endometriosis: Clinical characteristics and imaging findings. *Radiographics* 2018.

Chapron C, Lang JH, Leng JH, Zhou Y, Zhang X, Xue M, Popov A, Romanov V, Maisonobe P, Cabri P. Factors and Regional Differences Associated with Endometriosis: A Multi-Country, Case–Control Study. *Adv Ther* 2016.

Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. *Nat Rev Endocrinol* 2019.

Chapron C, Santulli P, De Ziegler D, Noel JC, Anaf V, Streuli I, Foulot H, Souza C, Borghese B. Ovarian endometrioma: Severe pelvic pain is associated with deeply infiltrating endometriosis. *Hum Reprod* 2012.

Chapron C, Tosti C, Marcellin L, Bourdon M, Lafay-Pillet MC, Millischer AE, Streuli I, Borghese B, Petraglia F, Santulli P. Relationship between the magnetic resonance imaging appearance of adenomyosis and endometriosis phenotypes. *Hum Reprod* 2017;**32**:1393–1401.

Chapron C, Vannuccini S, Santulli P, Abrão MS, Carmona F, Fraser IS, Gordts S, Guo SW, Just PA, Noël JC, *et al.* Diagnosing adenomyosis: An integrated clinical and imaging approach. *Hum Reprod Update* 2020.

Cheong Y, Tay P, Luk F, Gan HC, Li TC, Cooke I. Laparoscopic surgery for endometriosis: How often do we need to re-operate? *J Obstet Gynaecol (Lahore)* 2008.

Chiaffarino F, Cipriani S, Ricci E, Roncella E, Mauri PA, Parazzini F, Vercellini P. Endometriosis and inflammatory bowel disease: A systematic review of the literature. *Eur J Obstet Gynecol Reprod Biol* 2020.

Chiang HJ, Lin PY, Huang FJ, Kung FT, Lin YJ, Sung PH, Lan KC. The impact of previous ovarian surgery on ovarian reserve in patients with endometriosis. *BMC Womens Health* 2015.

Cho S, Jung JA, Lee Y, Kim HY, Seo SK, Choi YS, Lee JS, Lee BS. Postoperative levonorgestrel-releasing intrauterine system versus oral contraceptives after gonadotropin-releasing hormone agonist treatment for preventing endometrioma recurrence. *Acta Obstet Gynecol Scand* 2014.

Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* 2009;**5**.

Chung MK, Chung RP, Gordon D. Interstitial cystitis and endometriosis in patients with chronic pelvic pain: The “Evil Twins” syndrome. *JSLS* 2005.

Clemenza S, Sorbi F, Noci I, Capezzuoli T, Turrini I, Carriero C, Buffi N, Fambrini M, Petraglia F. From pathogenesis to clinical practice: Emerging medical treatments for endometriosis. *Best Pract Res Clin Obstet Gynaecol* 2018.

Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983.

Coxon L, Horne AW, Vincent K. Pathophysiology of endometriosis-associated pain: A review of pelvic and central nervous system mechanisms. *Best Pract Res Clin Obstet Gynaecol* 2018.

Cuevas M, Cruz ML, Ramirez AE, Flores I, Thompson KJ, Bayona M, Vernon MW, Appleyard CB. Stress

during development of experimental endometriosis influences nerve growth and disease progression. *Reprod Sci* 2018;**25**.

Cuevas M, Flores I, Thompson KJ, Ramos-Ortolaza DL, Torres-Reveron A, Appleyard CB. Stress exacerbates endometriosis manifestations and inflammatory parameters in an animal model. *Reprod Sci* 2012;**19**.

Culley L, Law C, Hudson N, Denny E, Mitchell H, Baumgarten M, Raine-Fenning N. The social and psychological impact of endometriosis on women's lives: A critical narrative review. *Hum Reprod Update* 2013.

Dai Y, Li X, Shi J, Leng J. A review of the risk factors, genetics and treatment of endometriosis in Chinese women: A comparative update. *Reprod Health* 2018.

Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC. The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *Neuroimage* 2009.

Dior UP, Nisbet D, Fung JN, Foster G, Healey M, Montgomery GW, Rogers PAW, Holdsworth-Carson SJ, Girling JE. The Association of Sonographic Evidence of Adenomyosis with Severe Endometriosis and Gene Expression in Eutopic Endometrium. *J Minim Invasive Gynecol* 2019.

DiVasta AD, Vitonis AF, Laufer MR, Missmer SA. Spectrum of symptoms in women diagnosed with endometriosis during adolescence vs adulthood. *Am J Obstet Gynecol* 2018.

Di Donato N, Bertoldo V, Montanari G, Zannoni L, Caprara G, Seracchioli R. Question mark form of uterus: A simple sonographic sign associated with the presence of adenomyosis. *Ultrasound Obstet Gynecol* 2015.

Di Donato N, Montanari G, Benfenati A, Leonardi D, Bertoldo V, Monti G, Raimondo D, Seracchioli R. Prevalence of adenomyosis in women undergoing surgery for endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2014;**181**:289–293.

Donnez J, Squifflet J. Complications, pregnancy and recurrence in a prospective series of 500 patients operated on by the shaving technique for deep rectovaginal endometriotic nodules. *Hum Reprod* 2010.

Dunselman GAJ, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A, *et al.* ESHRE guideline: Management of women with endometriosis. *Hum Reprod* 2014.

Eisenberg VH, Arbib N, Schiff E, Goldenberg M, Seidman DS, Soriano D. Sonographic Signs of Adenomyosis Are Prevalent in Women Undergoing Surgery for Endometriosis and May Suggest a Higher Risk of Infertility. *Biomed Res Int* 2017;**2017**:1–9. Available at: https://www.hindawi.com/journals/bmri/2017/8967803/.

Eriksen HLF, Gunnersen KF, Sørensen JA, Munk T, Nielsen T, Knudsen UB. Psychological aspects of endometriosis: Differences between patients with or without pain on four psychological variables. *Eur J Obstet Gynecol Reprod Biol* 2008.

Exacoustos C, Lazzeri L, Zupi E. Expert sonographers and surgeons are needed to manage deep infiltrating endometriosis. *Ultrasound Obstet Gynecol* 2017.

Exacoustos C, Malzoni M, Di Giovanni A, Lazzeri L, Tosti C, Petraglia F, Zupi E. Ultrasound mapping system for the surgical management of deep infiltrating endometriosis. *Fertil Steril* 2014.

Exacoustos C, Manganaro L, Zupi E. Imaging for the evaluation of endometriosis and adenomyosis. *Best Pract Res Clin Obstet Gynaecol* 2014;**28**:655–681.

Facchin F, Barbara G, Dridi D, Alberico D, Buggio L, Somigliana E, Saita E, Vercellini P. Mental health in women with endometriosis: Searching for predictors of psychological distress. *Hum Reprod* 2017.

Facchin F, Barbara G, Saita E, Mosconi P, Roberto A, Fedele L, Vercellini P. Impact of endometriosis on quality of life and mental health: Pelvic pain makes the difference. *J Psychosom Obstet Gynecol* 2015.

Falcone T, Flyckt-Rebecca R. Clinical management of endometriosis. *Obstet Gynecol* 2018.

Farland L V, Missmer SA, Bijon A, Gusto G, Gelot A, Clavel-Chapelon F, Mesrine S, Boutron-Ruault MC, Kvaskoff M. Associations among body size across the life course, adult height and endometriosis. *Hum Reprod* 2017;**32**.

Fauconnier A, Chapron C. Endometriosis and pelvic pain: Epidemiological evidence of the relationship and implications. *Hum Reprod Update* 2005.

Fawole AO, Bello FA, Ogunbode O, Odukogbe ATA, Nkwocha GC, Nnoaham KE, Zondervan KT, Akintan A, Abdus-Salam RA, Okunlola MA. Endometriosis and associated symptoms among Nigerian women. *Int J Gynecol Obstet* 2015.

Fedele L, Berlanda N, Corsi C, Gazzano G, Morini M, Vercellini P. Ileocecal endometriosis: Clinical and pathogenetic implications of an underdiagnosed condition. *Fertil Steril* 2014.

Filippi I, Carrarelli P, Luisi S, Batteux F, Chapron C, Naldini A, Petraglia F. Different Expression of Hypoxic and Angiogenic Factors in Human Endometriotic Lesions. *Reprod Sci* 2016.

Flores I, Abreu S, Abac S, Fourquet J, Laboy J, Ríos-Bedoya C. Self-reported prevalence of endometriosis and its symptoms among Puerto Rican women. *Int J Gynecol Obstet* 2008.

Florio P, Luisi S, Viganò P, Busacca M, Fadalti M, Genazzani AR, Petraglia F. Healthy women and patients with endometriosis show high concentrations of inhibin A, inhibin B, and activin A in peritoneal fluid throughout the menstrual cycle. *Hum Reprod* 1998.

Flyckt R, Kim S, Falcone T. Surgical Management of Endometriosis in Patients with Chronic Pelvic Pain. *Semin Reprod Med* 2017.

Fourquet J, Báez L, Figueroa M, Iriarte RI, Flores I. Quantification of the impact of endometriosis symptoms on health-related quality of life and work productivity. *Fertil Steril* 2011.

Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. *Psychoneuroendocrinology* 2005.

Friggi Sebe Petrelluzzi K, Garcia MC, Petta CA, Ribeiro DA, De Oliveira Monteiro NR, Céspedes IC, Spadari RC. Physical therapy and psychological intervention normalize cortisol levels and improve vitality in women with endometriosis. *J Psychosom Obstet Gynecol* 2012.

Frisch S. Perceptions of pain. Cultural differences add to the challenge of treating patients' pain. *Minn Med* 2014.

Frodl T, O'Keane V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. *Neurobiol Dis* 2013.

Fuentes IM, Christianson JA. The influence of early life experience on visceral pain. *Front Syst Neurosci* 2018;**12**.

Galhardo A, Moura-Ramos M, Cunha M, Pinto-Gouveia J. The infertility trap: How defeat and entrapment affect depressive symptoms. *Hum Reprod* 2016.

Gandek B, Ware JE, Aaronson NK, Apolone G, Bjorner JB, Brazier JE, Bullinger M, Kaasa S, Leplege A, Prieto L, *et al.* Cross-validation of item selection and scoring for the SF-12 Health Survey in nine countries: Results from the IQOLA Project. *J Clin Epidemiol* 1998.

Garavaglia E, Ricci E, Chiaffarino F. Leisure and occupational physical activity at different ages and risk of endometriosis. *Eur J Obs Gynecol Reprod Biol* 2014;**183**.

Garcia-Velasco JA, Arici A. Surgery for the removal of endometriomas before in vitro fertilization does not increase implantation and pregnancy rates. *Fertil Steril* 2004.

Ghai V, Jan H, Shakir F, Haines P, Kent A. Diagnostic delay for superficial and deep endometriosis in the United Kingdom. *J Obstet Gynaecol (Lahore)* 2020.

Godoy LD, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, Umeoka EH de L. A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. *Front Behav Neurosci* 2018.

Goetz LG, Mamillapalli R, Taylor HS. Low body mass index in endometriosis is promoted by hepatic metabolic gene dysregulation in mice. *Biol Reprod* 2016;**95**.

Goodman LR, Goldberg JM, Flyckt RL, Gupta M, Harwalker J, Falcone T. Effect of surgery on ovarian reserve in women with endometriomas, endometriosis and controls. In: *American Journal of Obstetrics and Gynecology*. 2016.

Gori M, Luddi A, Belmonte G, Piomboni P, Tosti C, Funghi L, Zupi E, Lazzeri L, Petraglia F. Expression of microtubule associated protein 2 and synaptophysin in endometrium: high levels in deep infiltrating endometriosis lesions. *Fertil Steril* 2016;**105**:435–443.

De Graaff AA, D'hooghe TM, Dunselman GAJ, Dirksen CD, Hummelshoj L, Simoens S, Bokor A, Brandes I, Brodszky V, Canis M, *et al.* The significant effect of endometriosis on physical, mental and social wellbeing: Results from an international cross-sectional survey. *Hum Reprod* 2013;**28**:2677–2685.

Greenbaum H i. l. a., Weil C l. a. r. a., Chodick G a. b. r. i. e. l., Shalev V a. r. d. a., Eisenberg V e. r. e. d. H. Evidence for an association between endometriosis, fibromyalgia, and autoimmune diseases. *Am J Reprod Immunol* 2019;**81**.

Greene AD, Lang SA, Kendziorski JA, Sroga-Rios JM, Herzog TJ, Burns KA. Endometriosis: Where are we and where are we going? *Reproduction* 2016.

Greff MJE, Levine JM, Abuzgaia AM, Elzagallaai AA, Rieder MJ, van Uum SHM. Hair cortisol analysis: An update on methodological considerations and clinical applications. *Clin Biochem* 2019.

Guerriero S, Ajossa S, Minguez JA, Jurado M, Mais V, Melis GB, Alcazar JL. Accuracy of transvaginal ultrasound for diagnosis of deep endometriosis in uterosacral ligaments, rectovaginal septum, vagina

and bladder: Systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2015.

Guerriero S, Condous G, van den Bosch T, Valentin L, Leone FPG, Van Schoubroeck D, Exacoustos C, Installé AJF, Martins WP, Abrao MS, *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.

Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol* 2007;**58**.

Guo SW. Recurrence of endometriosis and its control. *Hum Reprod Update* 2009.

Guo SW. Fibrogenesis resulting from cyclic bleeding: The Holy Grail of the natural history of ectopic endometrium. *Hum Reprod* 2018.

Guo SW, Zhang Q, Liu X. Social psychogenic stress promotes the development of endometriosis in mouse. *Reprod BioMed Online* 2017;**34**.

Han SJ, O'Malley BW. The dynamics of nuclear receptors and nuclear receptor coregulators in the pathogenesis of endometriosis. *Hum Reprod Update* 2014;**20**:467–484.

Harris HR, Costenbader KH, Mu F, Kvaskoff M, Malspeis S, Karlson EW, Missmer SA. Endometriosis and the risks of systemic lupus erythematosus and rheumatoid arthritis in the Nurses' health study II. *Ann Rheum Dis* 2016;**75**.

Harris HR, Wieser F, Vitonis AF, Rich-Edwards J, Boynton-Jarrett R, Bertone-Johnson ER, Missmer SA. Early life abuse and risk of endometriosis. *Hum Reprod* 2018;**33**.

Harrison V, Rowan K, Mathias J. Stress reactivity and family relationships in the development and treatment of endometriosis. *Fertil Steril* 2005.

Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. *Psychoneuroendocrinology* 2000;**25**.

Hernandez S, Cruz ML, Seguinot II, Torres-Reveron A, Appleyard CB. Impact of psychological stress on pain perception in an animal model of endometriosis. *Reprod Sci* 2017;**24**.

Van Holsbeke C, Van Calster B, Guerriero S, Savelli L, Paladini D, Lissoni AA, Czekierdowski A, Fischerova D, Zhang J, Mestdagh G, *et al.* Endometriomas: Their ultrasound characteristics. *Ultrasound Obstet Gynecol* 2010.

Horne AW, Daniels J, Hummelshoj L, Cox E, Cooper KG. Surgical removal of superficial peritoneal endometriosis for managing women with chronic pelvic pain: time for a rethink? *BJOG An Int J Obstet Gynaecol* 2019.

Howard FM. Endometriosis and Mechanisms of Pelvic Pain. *J Minim Invasive Gynecol* 2009;**16**:540–550.

Hu X, Zhou Y, Feng Q, Wang R, Su L, Long J, Wei B. Association of endometriosis risk and genetic polymorphisms involving biosynthesis of sex steroids and their receptors: An updating meta-analysis. *Eur J Obstet Gynecol Reprod Biol* 2012;**164**:1–9.

Hudelist G, Ballard K, English J, Wright J, Banerjee S, Mastoroudes H, Thomas A, Singer CF, Keckstein J. Transvaginal sonography vs. clinical examination in the preoperative diagnosis of deep infiltrating

endometriosis. *Ultrasound Obstet Gynecol* 2011.

Hudelist G, Oberwinkler KH, Singer CF, Tuttlies F, Rauter G, Ritter O, Keckstein J. Combination of transvaginal sonography and clinical examination for preoperative diagnosis of pelvic endometriosis. *Hum Reprod* 2009.

Ianieri MM, Mautone D, Ceccaroni M. Recurrence in Deep Infiltrating Endometriosis: A Systematic Review of the Literature. *J Minim Invasive Gynecol* 2018.

Janssen EB, Rijkers ACM, Hoppenbrouwers K, Meuleman C, D'Hooghe TM. Prevalence of endometriosis diagnosed by laparoscopy in adolescents with dysmenorrhea or chronic pelvic pain: A systematic review. *Hum Reprod Update* 2013.

Jess T, Frisch M, Jorgensen KT, Pedersen B V., Nielsen NM. Increased risk of inflammatory bowel disease in women with endometriosis: a nationwide Danish cohort study. *Gut* 2012;**61**:1279–1283. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22184069.

Jia SZ, Leng JH, Shi JH, Sun PR, Lang JH. Health-related quality of life in women with endometriosis: A systematic review. *J Ovarian Res* 2012.

Jones GT. Psychosocial vulnerability and early life adversity as risk factors for central sensitivity syndromes. *Curr Rheumatol Rev* 2016;**12**.

Kalantaridou SN, Zoumakis E, Makrigiannakis A, Lavasidis LG, Vrekoussis T, Chrousos GP. Corticotropin-releasing hormone, stress and human reproduction: An update. *J Reprod Immunol* 2010.

Keckstein J, Becker CM, Canis M, Feki A, Grimbizis GF, Hummelshoj L, Nisolle M, Roman H, Saridogan E, *et al.* Recommendations for the surgical treatment of endometriosis. Part 2: deep endometriosis †‡¶. *Hum Reprod Open* 2020.

Kho RM, Andres MP, Borrelli GM, Neto JS, Zanluchi A, Abrão MS. Surgical treatment of different types of endometriosis: Comparison of major society guidelines and preferred clinical algorithms. *Best Pract Res Clin Obstet Gynaecol* 2018.

Koga K, Takamura M, Fujii T, Osuga Y. Prevention of the recurrence of symptom and lesions after conservative surgery for endometriosis. *Fertil Steril* 2015.

Koninckx PR, Ussia A, Adamyan L, Wattiez A, Donnez J. Deep endometriosis: Definition, diagnosis, and treatment. *Fertil Steril* 2012.

Koshiba A, Mori T, Okimura H, Akiyama K, Kataoka H, Takaoka O, Ito F, Matsushima H, Kusuki I, Kitawaki J. Dienogest therapy during the early stages of recurrence of endometrioma might be an alternative therapeutic option to avoid repeat surgeries. *J Obstet Gynaecol Res* 2018.

Krizsan-Agbas D, Pedchenko T, Hasan W, Smith PG. Oestrogen regulates sympathetic neurite outgrowth by modulating brain derived neurotrophic factor synthesis and release by the rodent uterus. *Eur J Neurosci* 2003.

Küçükbaş M, Kurek Eken M, İlhan G, Şenol T, Herkiloğlu D, Kapudere B. Which factors are associated with the recurrence of endometrioma after cystectomy? *J Obstet Gynaecol (Lahore)* 2018.

Kuznetsov L, Dworzynski K, Davies M, Overton C. Diagnosis and management of endometriosis: summary

of NICE guidance. *BMJ* 2017:j3935.

Kvaskoff M, Mu F, Terry KL, Harris HR, Poole EM, Farland L, Missmer SA. Endometriosis: A high-risk population for major chronic diseases? *Hum Reprod Update* 2014;**21**:500–516.

Kvaskoff M, Mu F, Terry KL, Harris HR, Poole EM, Farland L, Missmer SA. Endometriosis: a high-risk population for major chronic diseases? *Hum Reprod Update* 2015;**21**:500–516..

Kwok W, Bhuvanakrishna T. The relationship between ethnicity and the pain experience of cancer patients: A systematic review. *Indian J Palliat Care* 2014.

Lafay Pillet MC, Schneider A, Borghese B, Santulli P, Souza C, Streuli I, de Ziegler D, Chapron C. Deep infiltrating endometriosis is associated with markedly lower body mass index: a 476 case-control study. *Hum Reprod* 2012;**27**.

Laganà AS, La Rosa VL, Rapisarda AMC, Valenti G, Sapia F, Chiofalo B, Rossetti D, Ban Frangež H, Vrtačnik Bokal E, Giovanni Vitale S. Anxiety and depression in patients with endometriosis: Impact and management challenges. *Int J Womens Health* 2017.

Lazzeri L, Di Giovanni A, Exacoustos C, Tosti C, Pinzauti S, Malzoni M, Petraglia F, Zupi E. Preoperative and Postoperative Clinical and Transvaginal Ultrasound Findings of Adenomyosis in Patients With Deep Infiltrating Endometriosis. *Reprod Sci* 2014;**21**:1027–1033.

Lazzeri L, Orlandini C, Vannuccini S, Pinzauti S, Tosti C, Zupi E, Nappi RE, Petraglia F. Endometriosis and perceived stress: Impact of surgical and medical treatment. *Gynecol Obstet Invest* 2015.

Lazzeri L, Vannuccini S, Orlandini C, Luisi S, Zupi E, Nappi RE, Petraglia F. Surgical treatment affects perceived stress differently in women with endometriosis: Correlation with severity of pain. *Fertil Steril* 2015a;**103**:433–438.

Lazzeri L, Vannuccini S, Orlandini C, Luisi S, Zupi E, Nappi RE, Petraglia F. Surgical treatment affects perceived stress differently in women with endometriosis: Correlation with severity of pain. *Fertil Steril* 2015b.

Lee DY, Kim E, Choi MH. Technical and clinical aspects of cortisol as a biochemical marker of chronic stress. *BMB Rep* 2015.

Levenstein S, Prantera C, Varvo V, Scribano ML, Berto E, Luzi C, Andreoli A. Development of the perceived stress questionnaire: A new tool for psychosomatic research. *J Psychosom Res* 1993.

Leyendecker G, Bilgicyildirim A, Inacker M, Stalf T, Huppert P, Mall G, Böttcher B, Wildt L. Adenomyosis and endometriosis. Re-visiting their association and further insights into the mechanisms of auto-traumatisation. An MRI study. *Arch Gynecol Obstet* 2015;**291**:917–932.

Leyland N, Casper R, Laberge P, Singh SS, SOGC. Endometriosis: diagnosis and management. *J Obstet Gynaecol Can* 2010;**32**:S1-32.

Li R, Sun X, Liu X, Yang Y, Li Z. Autoimmune diseases in China. In: *Advances in Immunology*. 2019.

Li Y, Hao N, Wang YX, Kang S. Association of Endometriosis-Associated Genetic Polymorphisms from Genome-Wide Association Studies with Ovarian Endometriosis in a Chinese Population. *Reprod Sci* 2017.

Liebermann C, Kohl Schwartz AS, Charpidou T, Geraedts K, Rauchfuss M, Wölfler M, Von Orelli S, Haberlin F, Eberhard M, Imesch P, *et al.* Maltreatment during childhood: A risk factor for the development of endometriosis? *Hum Reprod* 2018.

Lima AP, Moura MD, Rosa e Silva AAM. Prolactin and cortisol levels in women with endometriosis. *Brazilian J Med Biol Res* 2006.

Long Q, Liu X, Qi Q, Guo SW. Chronic stress accelerates the development of endometriosis in mouse through adrenergic receptor beta2. *Hum Reprod* 2016;**31**.

Lövkvist L, Boström P, Edlund M, Olovsson M. Age-related differences in quality of life in Swedish women with endometriosis. *J Women's Heal* 2016.

Luisi S, Pizzo A, Pinzauti S, Zupi E, Centini G, Lazzeri L, Di Carlo C, Petraglia F. Neuroendocrine and stress-related aspects of endometriosis. *Neuroendocrinol Lett* 2015.

Lynch CD, Sundaram R, Maisog JM, Sweeney AM, Buck Louis GM. Preconception stress increases the risk of infertility: results from a couple-based prospective cohort study--the LIFE study. *Hum Reprod* 2014;**29**.

Macer ML, Taylor HS. Endometriosis and Infertility. A Review of the Pathogenesis and Treatment of Endometriosis-associated Infertility. *Obstet Gynecol Clin North Am* 2012.

Maclaran K, Agarwal N, Odejinmi F. Co-Existence of Uterine Myomas and Endometriosis in Women Undergoing Laparoscopic Myomectomy: Risk Factors and Surgical Implications. *J Minim Invasive Gynecol* 2014.

Maia LM, Rocha AL, Puerto HL, Petraglia F, Reis FM. Plasma urocortin-1 as a preoperative marker of endometriosis in symptomatic women. *Gynecol Endocrinol* 2018;**34**.

Marinho MCP, Magalhaes TF, Fernandes LFC, Augusto KL, Brilhante AVM, Bezerra LRPS. Quality of Life in Women with Endometriosis: An Integrative Review. *J Women's Heal* 2018.

Márki G, Bokor A, Rigó J, Rigó A. Physical pain and emotion regulation as the main predictive factors of health-related quality of life in women living with endometriosis. *Hum Reprod* 2017.

Martin CE, Johnson E, Wechter ME, Leserman J, Zolnoun DA. Catastrophizing: a predictor of persistent pain among women with endometriosis at 1 year. *Hum Reprod* 2011;**26**.

McPeak AE, Allaire C, Williams C, Albert A, Lisonkova S, Yong PJ. Pain Catastrophizing and pain health-related quality-of-life in endometriosis. *Clin J Pain* 2018;**34**.

Missmer SA, Tu FF, Agarwal SK, Chapron C, Soliman AM, Chiuve S, Eichner S, Flores-Caldera I, Horne AW, Kimball AB, *et al.* Impact of Endometriosis on Life-Course Potential: A Narrative Review. *Int J Gen Med* 2021;**Volume 14**:9–25. Available at: https://www.dovepress.com/impact-of-endometriosis-on-life-course-potential-a-narrative-review-peer-reviewed-article-IJGM.

Montanari E, Dauser B, Keckstein J, Kirchner E, Nemeth Z, Hudelist G. Association between disease extent and pain symptoms in patients with deep infiltrating endometriosis. *Reprod Biomed Online* 2019.

Morotti M, Vincent K, Becker CM. Mechanisms of pain in endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2017.

Morotti M, Vincent K, Brawn J, Zondervan KT, Becker CM. Peripheral changes in endometriosis-associated pain. *Hum Reprod Update* 2014.

Muzii L, Galati G, Di Tucci C, Di Feliciantonio M, Perniola G, Di Donato V, Benedetti Panici P, Vignali M. Medical treatment of ovarian endometriomas: a prospective evaluation of the effect of dienogest on ovarian reserve, cyst diameter, and associated pain. *Gynecol Endocrinol* 2020.

Muzii L, Tucci C Di, Feliciantonio M Di, Galati G, Verrelli L, Donato V Di, Marchetti C, Panici PB. Management of Endometriomas. *Semin Reprod Med* 2017.

Naftalin J, Hoo W, Pateman K, Mavrelos D, Holland T, Jurkovic D. How common is adenomyosis? A prospective study of prevalence using transvaginal ultrasound in a gynaecology clinic. *Hum Reprod* 2012;**27**:3432–3439.

Naphatthalung W, Cheewadhanaraks S. Prevalence of endometriosis among patients with adenomyosis and/or myoma uteri scheduled for a hysterectomy. *J Med Assoc Thail* 2012.

Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 2009;**34**.

Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, *et al.* Incidence and phenotype of inflammatory bowel disease based on results from the Asia-Pacific Crohn's and colitis epidemiology study. *Gastroenterology* 2013.

Nielsen NM, Jorgensen KT, Pedersen B V, Rostgaard K, Frisch M. The co-occurrence of endometriosis with multiple sclerosis, systemic lupus erythematosus and Sjogren syndrome. *Hum Reprod* 2011;**26**.

Nirgianakis K, McKinnon B, Imboden S, Knabben L, Gloor B, Mueller MD. Laparoscopic management of bowel endometriosis: Resection margins as a predictor of recurrence. *Acta Obstet Gynecol Scand* 2014.

Nisenblat V, Bossuyt PMM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. *Cochrane Database Syst Rev* 2016.

Nnoaham KE, Hummelshoj L, Webster P, D'Hooghe T, De Cicco Nardone F, De Cicco Nardone C, Jenkinson C, Kennedy SH, Zondervan KT. Impact of endometriosis on quality of life and work productivity: A multicenter study across ten countries. *Fertil Steril* 2011a;**96**.

Nnoaham KE, Hummelshoj L, Webster P, D'Hooghe T, De Cicco Nardone F, De Cicco Nardone C, Jenkinson C, Kennedy SH, Zondervan KT. Impact of endometriosis on quality of life and work productivity: A multicenter study across ten countries. *Fertil Steril* 2011b.

Novembri R, Borges LE, Carrarelli P, Rocha ALL, De Pascalis F, Florio P, Petraglia F. Impaired CRH and urocortin expression and function in eutopic endometrium of women with endometriosis. *J Clin Endocrinol Metab* 2011;**96**:1145–1150.

Novembri R, Carrarelli P, Toti P, Rocha ALL, Borges LE, Reis FM, Piomboni P, Florio P, Petraglia F. Urocortin 2 and urocortin 3 in endometriosis: evidence for a possible role in inflammatory response. *Mol Hum Reprod* 2011;**17**:587–593.

Nowak-Psiorz I, Ciećwież SM, Brodowska A, Starczewski A. Treatment of ovarian endometrial cysts in the context of recurrence and fertility. *Adv Clin Exp Med* 2019.

Parazzini F, Esposito G, Tozzi L, Noli S, Bianchi S. Epidemiology of endometriosis and its comorbidities. *Eur J Obstet Gynecol Reprod Biol* 2017.

Patel BG, Lenk EE, Lebovic DI, Shu Y, Yu J, Taylor RN. Pathogenesis of endometriosis: Interaction between Endocrine and inflammatory pathways. *Best Pract Res Clin Obstet Gynaecol* 2018.

Petraglia F, Imperatore A, Challis JRG. Neuroendocrine mechanisms in pregnancy and parturition. *Endocr Rev* 2010;**31**:783–816.

Petrelluzzi KFS, Garcia MC, Petta CA, Grassi-Kassisse DM, Spadari-Bratfisch RC. Salivary cortisol concentrations, stress and quality of life in women with endometriosis and chronic pelvic pain. *Stress* 2008.

Pluchino N, Wenger JM, Petignat P, Tal R, Bolmont M, Taylor HS, Bianchi-Demicheli F. Sexual function in endometriosis patients and their partners: Effect of the disease and consequences of treatment. *Hum Reprod Update* 2016.

Practice bulletin no. 114: Management of endometriosis. *Obstet Gynecol* 2010.

Quiñones M, Urrutia R, Torres-Reverón A, Vincent K, Flores I. Anxiety, coping skills and hypothalamus-pituitary-adrenal (HPA) axis in patients with endometriosis. *J Reprod Biol Heal* 2015.

Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: A systematic review and meta-analysis. *J Clin Endocrinol Metab* 2012.

Rafique S, Decherney AH. Medical Management of Endometriosis. *Clin Obstet Gynecol* 2017.

Redwine DB. Diaphragmatic endometriosis: diagnosis, surgical management, and long-term results of treatment. *Fertil Steril* 2002.

Reis FM, Coutinho LM, Vannuccini S, Batteux F, Chapron C, Petraglia F. Progesterone receptor ligands for the treatment of endometriosis: The mechanisms behind therapeutic success and failure. *Hum Reprod Update* 2020.

Reis FM, Coutinho LM, Vannuccini S, Luisi S, Petraglia F. Is Stress a Cause or a Consequence of Endometriosis? *Reprod Sci* 2020.

Reis FM, Petraglia F, Taylor RN. Endometriosis: Hormone regulation and clinical consequences of chemotaxis and apoptosis. *Hum Reprod Update* 2013;**19**:406–418.

Riccio L da GC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. *Best Pract Res Clin Obstet Gynaecol* 2018.

Rocha AL, Vieira EL, Ferreira MC, Maia LM, Teixeira AL, Reis FM. Plasma brain-derived neurotrophic factor in women with pelvic pain: a potential biomarker for endometriosis? *Biomark Med* 2017;**11**.

Rocha ALL, Reis FM, Petraglia F. New trends for the medical treatment of endometriosis. *Expert Opin Investig Drugs* 2012.

Römer T. Long-term treatment of endometriosis with dienogest: retrospective analysis of efficacy and safety in clinical practice. *Arch Gynecol Obstet* 2018.

Santulli P, Lamau MC, Marcellin L, Gayet V, Marzouk P, Borghese B, Lafay Pillet MC, Chapron C. Endometriosis-related infertility: Ovarian endometrioma per se is not associated with presentation for

infertility. *Hum Reprod* 2016.

Saraswat L, Ayansina D, Cooper KG, Bhattacharya S, Horne AW, Bhattacharya S. Impact of endometriosis on risk of further gynaecological surgery and cancer: a national cohort study. *BJOG An Int J Obstet Gynaecol* 2018.

Saridogan E, Becker CM, Feki A, Grimbizis GF, Hummelshoj L, Keckstein J, Nisolle M, Tanos V, Ulrich UA, *et al.* Recommendations for the Surgical Treatment of Endometriosis. Part 1: Ovarian Endometrioma†‡¶. *Hum Reprod Open* 2017.

Saridogan E, Becker CM, Feki A, Grimbizis GF, Hummelshoj L, Keckstein J, Nisolle M, Tanos V, Ulrich UA, Vermeulen N, *et al.* Recommendations for the surgical treatment of endometriosis—part 1: ovarian endometrioma. *Gynecol Surg* 2017.

Lo Sauro C, Ravaldi C, Cabras PL, Faravelli C, Ricca V. Stress, hypothalamic-pituitary-adrenal axis and eating disorders. *Neuropsychobiology* 2008;**57**.

Schliep KC, Mumford SL, Peterson CM, Chen Z, Johnstone EB, Sharp HT, Stanford JB, Hammoud AO, Sun L, Buck Louis GM. Pain typology and incident endometriosis. *Hum Reprod* 2015.

Selcuk S, Cam C, Koc N, Kucukbas M, Ozkaya E, Eser A, Karateke A. Evaluation of risk factors for the recurrence of ovarian endometriomas. *Eur J Obstet Gynecol Reprod Biol* 2016.

Selye H. A sundrome produced by nocuous agents. *Nature* 1936.

Selye H. Stress and the general adaptation syndrome. *Br Med J* 1950.

Seo JW, Lee DY, Yoon BK, Choi DS. The Efficacy of Postoperative Cyclic Oral Contraceptives after Gonadotropin-Releasing Hormone Agonist Therapy to Prevent Endometrioma Recurrence in Adolescents. *J Pediatr Adolesc Gynecol* 2017.

Sepulcri Rde P. Do Amaral VF. Depressive symptoms, anxiety, and quality of life in women with pelvic endometriosis. *Eur J Obs Gynecol Reprod Biol* 2009;**142**.

Shah DK, Correia KF, Vitonis AF, Missmer SA. Body size and endometriosis: results from 20 years of follow-up within the Nurses' health study II prospective cohort. *Hum Reprod* 2013;**28**.

Shakiba K, Bena JF, McGill KM, Minger J, Falcone T. Surgical treatment of endometriosis: a 7-year follow-up on the requirement for further surgery. *Obstet Gynecol* 2008.

Shigesi N, Kvaskoff M, Kirtley S, Feng Q, Fang H, Knight JC, Missmer SA, Rahmioglu N, Zondervan KT, Becker CM. The association between endometriosis and autoimmune diseases: A systematic review and meta-analysis. *Hum Reprod Update* 2019.

Sibiude J, Santulli P, Marcellin L, Borghese B, Dousset B, Chapron C. Association of history of surgery for endometriosis with severity of deeply infiltrating endometriosis. *Obstet Gynecol* 2014.

Siedentopf F, Tariverdian N, Rücke M, Kentenich H, Arck PC. Immune status, psychosocial distress and reduced quality of life in infertile patients with endometriosis. *Am J Reprod Immunol* 2008.

Simoens S, Dunselman G, Dirksen C, Hummelshoj L, Bokor A, Brandes I, Brodszky V, Canis M, Colombo GL, Deleire T, *et al.* The burden of endometriosis: Costs and quality of life of women with endometriosis and treated in referral centres. *Hum Reprod* 2012.

Singh SS, Suen MWH. Surgery for endometriosis: beyond medical therapies. *Fertil Steril* 2017.

Soliman AM, Coyne KS, Gries KS, Castelli-Haley J, Snabes MC, Surrey ES. The effect of endometriosis symptoms on absenteeism and presenteeism in the workplace and at home. *J Manag Care Spec Pharm* 2017.

Soliman AM, Fuldeore M, Snabes MC. Factors Associated with Time to Endometriosis Diagnosis in the United States. *J Women's Heal* 2017.

Soliman AM, Yang H, Du EX, Kelley C, Winkel C. The direct and indirect costs associated with endometriosis: A systematic literature review. *Hum Reprod* 2016.

Somigliana E, Busnelli A, Benaglia L, Viganò P, Leonardi M, Paffoni A, Vercellini P. Postoperative hormonal therapy after surgical excision of deep endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2017.

Somigliana E, Vercellini P, Vigano P, Benaglia L, Busnelli A, Fedele L. Postoperative Medical Therapy After Surgical Treatment of Endometriosis: From Adjuvant Therapy to Tertiary Prevention. *J Minim Invasive Gynecol* 2014.

Staal AHJ, Van Der Zanden M, Nap AW. Diagnostic Delay of Endometriosis in the Netherlands. *Gynecol Obstet Invest* 2016;**81**:321–324.

Stratton P, Berkley KJ. Chronic pelvic pain and endometriosis: Translational evidence of the relationship and implications. *Hum Reprod Update* 2011.

Su SY, Muo CH, Sung FC, Morisky DE. Reduction of surgery rate in endometriosis patients who take Chinese medicine: A population-based retrospective cohort study. *Complement Ther Med* 2014.

Surrey ES, Soliman AM, Johnson SJ, Davis M, Castelli-Haley J, Snabes MC. Risk of Developing Comorbidities Among Women with Endometriosis: A Retrospective Matched Cohort Study. *J Women's Heal* 2018.

Tafet GE, Nemeroff CB. The links between stress and depression: psychoneuroendocrinological, genetic, and environmental interactions. *J Neuropsychiatry Clin Neurosci* 2016;**28**.

Takaesu Y, Nishi H, Kojima J, Sasaki T, Nagamitsu Y, Kato R, Isaka K. Dienogest compared with gonadotropin-releasing hormone agonist after conservative surgery for endometriosis. *J Obstet Gynaecol Res* 2016.

Tanbo T, Fedorcsak P. Endometriosis-associated infertility: aspects of pathophysiological mechanisms and treatment options. *Acta Obstet Gynecol Scand* 2017.

Tanmahasamut P, Noothong S, Sanga-Areekul N, Silprasit K, Dangrat C. Prevalence of endometriosis in women undergoing surgery for benign gynecologic diseases. *J Med Assoc Thail* 2014.

Tariverdian N, Theoharides TC, Siedentopf F, Gutiérrez G, Jeschke U, Rabinovich GA, Blois SM, Arck PC. Neuroendocrine-immune disequilibrium and endometriosis: an interdisciplinary approach. *Semin Immunopathol* 2007;**29**.

Taylor E, Williams C. Surgical treatment of endometriosis: location and patterns of disease at reoperation. *Fertil Steril* 2010.

Teng SW, Horng HC, Ho CH, Yen MS, Chao HT, Wang PH, Chang YH, Chang Y, Chao KC, Chen YJ, *et al.* Women with endometriosis have higher comorbidities: Analysis of domestic data in Taiwan. *J Chinese Med Assoc* 2016.

The World Health Organization quality of life assessment (WHOQOL): Position paper from the World Health Organization. *Soc Sci Med* 1995.

Tobiume T, Kotani Y, Takaya H, Nakai H, Tsuji I, Suzuki A, Mandai M. Determinant factors of postoperative recurrence of endometriosis: difference between endometrioma and pain. *Eur J Obstet Gynecol Reprod Biol* 2016.

Tokushige N, Markham R, Russell P, Fraser IS. High density of small nerve fibres in the functional layer of the endometrium in women with endometriosis. *Hum Reprod* 2006.

Tomassetti C, D'Hooghe T. Endometriosis and infertility: Insights into the causal link and management strategies. *Best Pract Res Clin Obstet Gynaecol* 2018.

Tomassetti C, Meuleman C, Timmerman D, D'Hooghe T. Adenomyosis and Subfertility: Evidence of Association and Causation. *Semin Reprod Med* 2013;**31**:101–108. Available at: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0032-1333475.

Tosti C, Pinzauti S, Santulli P, Chapron C, Petraglia F. Pathogenetic Mechanisms of Deep Infiltrating Endometriosis. *Reprod Sci* 2015;**22**:1053–1059.

Touboul C, Amate P, Ballester M, Bazot M, Fauconnier A, Daraï E. Quality of Life Assessment Using EuroQOL EQ-5D Questionnaire in Patients with Deep Infiltrating Endometriosis: The Relation with Symptoms and Locations. *Int J Chronic Dis* 2013.

Treatment of pelvic pain associated with endometriosis: A committee opinion. *Fertil Steril* 2014.

Uimari O, Järvelä I, Ryynänen M. Do symptomatic endometriosis and uterine fibroids appear together. *J Hum Reprod Sci* 2011.

Upson K, Sathyanarayana S, Scholes D, Holt VL. Early-life factors and endometriosis risk. *Fertil Steril* 2015;**104**:964-971e5.

Vamvakopoulos NC, Chrousos GP. Evidence of direct estrogenic regulation of human corticotropin-releasing hormone gene expression potential implications for the sexual dimophism of the stress response and immune/inflammatory reaction. *J Clin Invest* 1993.

Vannuccini S, Lazzeri L, Orlandini C, Morgante G, Bifulco G, Fagiolini A, Petraglia F. Mental health, pain symptoms and systemic comorbidities in women with endometriosis: a cross-sectional study. *J Psychosom Obstet Gynecol* 2017.

Vannuccini S, Lazzeri L, Orlandini C, Morgante G, Bifulco G, Fagiolini A, Petraglia F. Mental health, pain symptoms and systemic comorbidities in women with endometriosis: a cross-sectional study. *J Psychosom Obs Gynaecol* 2018;**39**.

Vannuccini S, Lazzeri L, Orlandini C, Tosti C, Clifton VL, Petraglia F. Potential influence of in utero and early neonatal exposures on the later development of endometriosis. *Fertil Steril* 2016;**105**:997–1002.

Vannuccini S, Petraglia F. Recent advances in understanding and managing adenomyosis. *F1000Research*

2019.

Vannuccini S, Reis FM, Coutinho LM, Lazzeri L, Centini G, Petraglia F. Surgical treatment of endometriosis: prognostic factors for better quality of life. *Gynecol Endocrinol* 2019;**35**.

Vannuccini S, Tosti C, Carmona F, Huang SJ, Chapron C, Guo S-W, Petraglia F. Pathogenesis of adenomyosis: An update on molecular mechanisms. *Reprod Biomed Online* 2017.

Vercellini P, Barbara G, Abbiati A, Somigliana E, Viganò P, Fedele L. Repetitive surgery for recurrent symptomatic endometriosis: What to do? *Eur J Obstet Gynecol Reprod Biol* 2009.

Vercellini P, Bracco B, Mosconi P, Roberto A, Alberico D, Dhouha D, Somigliana E. Norethindrone acetate or dienogest for the treatment of symptomatic endometriosis: A before and after study. *Fertil Steril* 2016.

Vercellini P, Buggio L, Berlanda N, Barbara G, Somigliana E, Bosari S. Estrogen-progestins and progestins for the management of endometriosis. *Fertil Steril* 2016.

Vercellini P, Buggio L, Frattaruolo MP, Borghi A, Dridi D, Somigliana E. Medical treatment of endometriosis-related pain. *Best Pract Res Clin Obstet Gynaecol* 2018.

Vercellini P, Crosignani PG, Abbiati A, Somigliana E, Viganò P, Fedele L. The effect of surgery for symptomatic endometriosis: The other side of the story. *Hum Reprod Update* 2009.

Vercellini P, Frontino G, De Giorgi O, Pietropaolo G, Pasin R, Crosignani PG. Endometriosis preoperative and postoperative medical treatment. *Obstet Gynecol Clin North Am* 2003.

Vercellini P, Somigliana E, Viganò P, Abbiati A, Barbara G, Crosignani PG. Surgery for endometriosis-associated infertility: A pragmatic approach. *Hum Reprod* 2009.

Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 2014;**10**:261–275. Available at: http://www.nature.com/articles/nrendo.2013.255.

Videlock EJ, Adeyemo M, Licudine A. Childhood trauma is associated with hypothalamic-pituitary-adrenal axis responsiveness in irritable bowel syndrome. *Gastroenterology* 2009;**137**.

Vitonis AF, Baer HJ, Hankinson SE, Laufer MR, Missmer SA. A prospective study of body size during childhood and early adulthood and the incidence of endometriosis. *Hum Reprod* 2010;**25**.

Vitonis AF, Maruti SS, Hankinson SE, Hornstein MD, Missmer SA. Adolescent physical activity and endometriosis risk. *J Endometr* 2009;**1**.

Vlahos NF, Theodoridis TD, Partsinevelos GA. Myomas and Adenomyosis: Impact on Reproductive Outcome. *Biomed Res Int* 2017.

Wang G, Tokushige N, Markham R, Fraser IS. Rich innervation of deep infiltrating endometriosis. *Hum Reprod* 2009.

Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (Sf-36): I. conceptual framework and item selection. *Med Care* 1992.

Woolf CJ. Central sensitization: Implications for the diagnosis and treatment of pain. *Pain* 2011.

Wu B, Yang Z, Tobe RG, Wang Y. Medical therapy for preventing recurrent endometriosis after conservative surgery: a cost-effectiveness analysis. *BJOG An Int J Obstet Gynaecol* 2018.

Yen CF, Kim MR, Lee CL. Epidemiologic factors associated with endometriosis in East Asia. *Gynecol Minim Invasive Ther* 2019.

Yin B, Liu X, Guo SW. Caloric restriction dramatically stalls lesion growth in mice with induced endometriosis. *Reprod Sci* 2018;**25**.

Yudell M, Roberts D, DeSalle R, Tishkoff S. Science and society: Taking race out of human genetics. *Science (80-)* 2016.

Zarbo C, Brugnera A, Frigerio L, Malandrino C, Rabboni M, Bondi E, Compare A. Behavioral, cognitive, and emotional coping strategies of women with endometriosis: a critical narrative review. *Arch Womens Ment Health* 2018.

Zepiridis LI, Grimbizis GF, Tarlatzis BC. Infertility and uterine fibroids. *Best Pract Res Clin Obstet Gynaecol* 2016.

Zhao RH, Hao ZP, Zhang Y, Lian FM, Sun WW, Liu Y, Wang R, Long L, Cheng L, Ding YF, *et al.* Controlling the recurrence of pelvic endometriosis after a conservative operation: Comparison between Chinese herbal medicine and western medicine. *Chin J Integr Med* 2013.

Zhao RH, Liu Y, Tan Y, Hao ZP, Meng QW, Wang R, Long D, Ding YF, Song DR, Xu C, *et al.* Chinese medicine improves postoperative quality of life in endometriosis patients: A randomized controlled trial. *Chin J Integr Med* 2013.

Zheng Q, Mao H, Xu Y, Zhao J, Wei X, Liu P. Can postoperative GnRH agonist treatment prevent endometriosis recurrence? A meta-analysis. *Arch Gynecol Obstet* 2016.

Zhu S, Liu D, Huang W, Wang Q, Wang Q, Zhou L, Feng G. Post-laparoscopic oral contraceptive combined with Chinese herbal mixture in treatment of infertility and pain associated with minimal or mild endometriosis: A randomized controlled trial. *BMC Complement Altern Med* 2014.

De Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: Pathophysiology and management. *Lancet* 2010.

Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Vigano P. Endometriosis. *Nat Rev Dis Prim* 2018;**4**.

Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. *Nat Rev Dis Prim* 2018;**4**:9.

Zondervan KT, Becker CM, Missmer SA. Endometriosis. Longo DL (ed). *N Engl J Med* 2020;**382**:1244–1256.