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Aromatase inhibitors for the treatment of endometriosis: a systematic review about efficacy, safety and early clinical development

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Abstract

Introduction: Pharmacotherapy has a key role in endometriosis treatment and management, however, a significant proportion of patients have only intermittent or limited benefits with current treatment options. Therefore, novel therapeutic approaches are necessary.

Areas covered: This systematic review provides an overview of the efficacy and safety of aromatase inhibitors (AIs) as monotherapies and combination therapies for endometriosis. A systematic literature search was performed from January 1990 to April 2020 in the electronic database MEDLINE, EMBASE, The Cochrane Library, and Web of Science.

Expert opinion: Based on the critical role of estrogens and the rate-limiting step in the production of the estrogens represented by the aromatase enzyme, AIs are a potential therapeutic option for women affected by endometriosis. Nevertheless, further research is needed to clarify the efficacy of AIs in this setting. Adverse effects need to be investigated to clarify the preventive role of add-back therapy. On that basis, AIs should be adopted only as second-line therapy in patients who are refractory to standard treatments in the setting of scientific research. Further studies should define best dosages, appropriate add-back therapies, administration routes, treatment length, and which patients may benefit more from AIs.

Keywords: Endometriosis; Estrogen; Aromatase; Aromatase inhibitors; Dysmenorrhea; Dyschezia; Chronic pelvic pain; Adverse effects.
Article Highlights:

- Pharmacotherapy has a key role in the treatment of endometriosis; however, a significant proportion of patients have only intermittent or limited benefit with progestins/estroprogestins and GnRH analogs. Therefore, the development of other therapeutic options is necessary.
- Estrogens have a key role in the pathophysiology of endometriosis. Aromatase inhibitors (AIs) have been proposed to treat endometriosis, reducing ovarian, systemic, and local estrogen production.
- Fifteen clinical trials have investigated anastrozole and letrozole to treat endometriosis; however, the evidence related to their use is limited.
- Hormone add-back therapy during AI administration seems to prevent the reduction of bone mineral density and the development of functional cysts. However, the addition of AIs to hormone therapy has been associated with a higher rate of menopausal symptoms.
- Recent trials investigated the vaginal route of AI administration. Further research should define best dosages, appropriate add-back therapies, new administration routes, treatment length and those patients that may benefit most from AIs.
1. Introduction

Endometriosis is a chronic hormone-dependent inflammatory disease defined by the presence of ectopic endometrial tissue [1]. It mainly affects women of fertile age, with a prevalence estimated between 5 and 10% [2–4]. The clinical presentation of endometriosis is heterogeneous, primarily consisting of pain symptoms (dysmenorrhea, chronic pelvic pain [CPP], dyschezia, and dyspareunia) and infertility [5]. Pain symptoms and infertility are not always correlated with the anatomic disruption potentially due to ectopic implants [6]; many factors seem to be involved in the clinical presentation of endometriosis [7–10], and the planning of the most appropriate treatment for each patient is challenging [11].

Surgery has a critical role in managing the most severe cases of endometriosis (stage III or IV, according to the American Society of Reproductive Medicine [ASRM] classification) and disease-related complications, such as bowel [12–14] or ureteral occlusions [15,16]. Conversely, the role of surgery is less clear and more debated for the specific treatment of infertility and pain symptoms [17]. The complete postoperative resolution of symptoms can be not absolute, and fertility improvement is controversial [15,18–21]. Moreover, the disease tends to recur, in particular, when postoperative pharmacological therapy is not implemented [22].

When the desire of conception is not present, and surgery is not considered or has been already adopted, medical therapy represents the primary option to manage symptoms and, ideally, prevent disease progression or postoperative recurrence [11]. In addition to non-steroidal anti-inflammatory drugs (NSAIDs) used to control pain symptoms, the growing body of evidence about the multiple mechanisms involved in the development, survival, and progression of endometriotic implants is increasing the number of possible medications targeting the endometriosis etiopathogenesis [23–27]. Although some non-hormonal drugs are under early clinical investigation in phase I or phase II trials, the pharmacotherapy of endometriosis is mainly based on hormonal options, aiming to suppress the ovarian function and control the amount and type of hormones that achieve the endometriotic implants [23]. All hormone therapies aim to reduce or block the promoting action of estrogens or increase the inhibitory activity of progesterone [24], despite the widely documented progesterone-resistance of the disease.

Among current non-experimental options, progestins and estroprogestins are the first-line therapy and have been extensively investigated to treat women with endometriosis [11]. These compounds may be
administered by different routes and have been reported effective in controlling symptoms, particularly dysmenorrhea, with limited side effects [28–31]. These drugs act with negative feedback on gonadotropin-releasing hormone (GnRH) secretion, with gonadotropin levels reduction and subsequent ovarian function suppression. The induced hypoestrogenic state by progestins in monotherapy and the increased progesterone levels determined by both options cause the atrophy and decidualization of eutopic and ectopic endometrium [32], with multiple actions on endometriotic implants, among which a decreased secretion of pro-inflammatory mediators [24,33,34]. Alternatively, GnRH analogs are used to suppress ovarian function by blocking the release of gonadotropins at the pituitary level [35]. They provoke a hypoestrogenic state that has been reported effective in controlling endometriosis symptoms and able to induce the regression of endometriotic implants [36]. These drugs have the advantage of being available in long-acting formulations, which allow administering the GnRH analog up to every three months. In this regard, the long-acting formulation is now available even for GnRH antagonists with degarelix, which has been investigated in assisted reproduction and could be a suitable option in endometriosis [37]. Nevertheless, the induced hypoestrogenism by GnRH analogs causes significant adverse effects (AEs), such as depression, hot flushes, urogenital atrophy, and loss of body mineral density (BMD) [38]. On that basis, GnRH analogs, combined with add-back therapy, represent the second-line option in patients not responsive to progestins and estroprogestins [11].

The most relevant limit of available pharmacotherapy is that a significant proportion of patients have only intermittent or limited benefit with both progestins and estroprogestins, as well as with GnRH analogs [11]. Respectively, almost 30-50% of patients do not report a reduction or resolution of pain symptoms after first-line therapy. Moreover, a median of 5–25% of patients discontinues the treatment due to AEs or lack of efficacy [39]. Therefore, the investigation of other therapeutic options is mandatory to increase the available strategies and personalize the management of women affected by endometriosis. In this regard, aromatase inhibitors (AIs) represent a hormone therapy under investigation for clinical application in these patients [23].

2. Endometriosis, estrogens, and aromatase
Estrogens have a crucial role in the pathophysiology of endometriosis [24,40]. In both the eutopic and ectopic endometrium, the estrogen receptors $\alpha$ (ER-$\alpha$) and $\beta$ (ER-$\beta$) have been identified [41–43]. ER-$\alpha$ and ER-$\beta$ are two isoforms with around 55% of homology; both ERs contain a DNA binding region, a dimerization region, a ligand-binding domain, and two transactivation domains, which are involved in non-genomic pathways [44].

Nuclear receptors, present in the cytosol in an unliganded state, enter the nucleus after binding to hormone ligands, which determines conformational changes, chaperone proteins dissociation, dimerization, and exposure of the two activating function sites. ER-$\alpha$ and ER-$\beta$ mainly act as transcriptional factors regulating the expression of a specific subset of genes, which tend to be not redundant because of the different expression patterns and functions [41–43]. They have different roles mediated by different targeted genes, non-genomic pathways, affinity for specific estrogens, and tissue distribution of homodimers and heterodimers. These differences have regulatory functions, in which is involved a reciprocal inhibition [45].

The formation of homodimers and heterodimers is related to the expression level of the two isoforms; the heterodimer has a similar binding affinity to DNA as the ER-$\alpha$ homodimer, but a lower level of transcriptional activity [46].

ER-$\alpha$ is the primary mediator of estrogens in the eutopic endometrium, regulating regeneration and proliferation in the proliferative phase of the menstrual cycle [47,48]. ER-$\alpha$ regulate the expression and function of cell-cycle related genes, such as cyclins and cyclin-dependent kinases (CDK), determining the proliferation of endometrial cells [49]. At the same time, ER-$\alpha$ is involved in modulating and inhibiting the regeneration of the eutopic endometrium, such as the estrogen-mediated induction of progesterone receptors expression, which sensitizes endometrial cell to the action of progesterone [45].

As compared to the eutopic endometrium, endometriotic tissue seems to have a similar or lower expression of ER-$\alpha$ but overexpression of ER-$\beta$, with an inverted ER-$\beta$/ER-$\alpha$ ratio [43,45,48,50]. The higher expression of ER-$\beta$ in the endometriotic tissue, regulated by epigenetic mechanisms, and the overactivation of related pathways appear inhibiting the expression and activity of ER-$\alpha$ and related signaling; this may due to the loss of those mechanisms regulating the eutopic endometrium mediated by ER-$\alpha$, such as the estrogen-mediated induction of progesterone receptors [48]. This dysregulation and hyperactivation of ER-$\beta$ signaling may improve cell survival, escape from the immune system [51–53], and the function of the radical
oxygen species detoxification system [54]. Moreover, ER-\(\beta\) signaling may be involved in the invasion and progression of endometriotic implants [54].

The role of estrogens is further supported by epidemiological and clinical studies, which demonstrated a strong association between endometriosis and reproductive life, with the regression of endometriotic implants and associated symptomatology after menopause [2–4]. Indeed, the primary source of estrogens is represented by the ovaries, which cyclically produce estrogens during reproductive life. Luteinizing hormone stimulates the theca production of androgens from cholesterol, which diffuse to the granulosa cell layer of the follicle. In the granulosa cells, the follicle-stimulating hormone regulates the expression of the aromatase P450 enzyme, which is responsible for the transformation of androgens into estrogens [54]. Aromatase is a mono-oxygenase enzyme complex that aromatizes androstenedione and testosterone into estrone and estradiol, respectively, through three hydroxylation reactions. The A ring of the androgens is rearranged in a phenolic A ring, characteristic of estrogens [55]. In the ovaries, hormone production involves mainly the aromatization of testosterone into estradiol, which is the principal and more active female sex hormone. The estradiol reaches the endometriotic implants through the blood circulation or by direct spillage in the peritoneal cavity at ovulation [56]. The estrogen dependence of endometriosis and this primary source of estrogens explains the efficacy of all the pharmacotherapeutic options inhibiting and suppressing the ovarian function by blocking gonadotropins release [29,34–36].

Nevertheless, ovaries are not the unique estrogen production site; any tissue expressing aromatase P450 can convert androgens to estrogens. This enzyme is physiologically expressed in different human tissues, such as adipose tissue, skin, and skeletal muscle [57–59]. In these peripheral tissues, estrogens production involves mainly the aromatization of the circulating androstenedione of adrenal origin into estrone, which can be converted into estradiol at the level of endometriotic implants. This mechanism has been proposed as a possible additional source of estrogens, which may explain cases not responsive to ovarian function suppression [40].

However, in addition to peripheral tissues, the strong interest in the extra-ovarian aromatase function derives from the aromatase identification in endometriotic implants [60]. Polymerase chain reaction and immunohistochemistry studies reported the presence of aromatase in both the eutopic and ectopic endometrium of affected patients but its absence in the eutopic endometrium of healthy women [57–59,61–
63]. Moreover, the nuclear receptor SF-1, regulator of aromatase production, seems to be overexpressed in endometriosis [64]. The presence of aromatase in endometriotic implants represents a local source of estrogens, and it may justify the higher concentration of estradiol in the endometriotic tissue and the peritoneal and endometrioma fluid [65–69]. Moreover, aromatase and the local production of estrogen have been proposed involved in a positive feedback loop [45]. Estrogens induce the expression of the cyclooxygenase type 2 (COX-2) enzyme that elevates the prostaglandin E2 levels, which stimulates the aromatase activity by inducing the nuclear receptor SF-1 [61,64,70].

Based on these pieces of evidence, AIs have been proposed as hormone therapy in women affected by endometriosis, being able to suppress estrogen production at the level of ovaries, peripheral tissues, and endometriotic implants. However, more recent studies did not identify aromatase mRNA at the implant level, questioning the specific advantage of targeting the in situ estrogen production [71–73]. Nevertheless, the potential clinical value of AIs persists as a possible alternative or additional option to other hormone therapies. The direct inhibition of estrogen production by blocking the aromatase can provide a higher suppression of estrogen levels than an indirect inhibition through the ovarian function [59,74]. Noteworthy, following the example of AIs, new molecules targeting other enzymes involved in the estrogen biosynthesis, such as steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase 1 (17β-HSD-1), have been investigated [75].

3. Aromatase inhibitors and endometriosis

Three third-generation AIs are available in pill form; these drugs are reported having similar efficacy, despite having different molecular structure and pharmacologic properties [76]. Exemestane belongs to the type I steroidal Ais; being substrates of aromatase, they directly compete with androgens for the enzyme active-site and covalently bind it, causing irreversible inactivation. Letrozole and anastrozole, instead, belongs to the type II non-steroidal Ais; they are triazoles that bind reversibly to the aromatase, by competing with androgens for the binding sites [77].

Third-generation AIs are currently considered the first line of treatment for ER-positive breast cancer in postmenopausal and pre-menopausal women after ovaries ablation [78–81]. AIs were reported to provide a
significant reduction of estrogen levels with reduced cancer mortality compared to tamoxifen [59]. On that basis, the majority of experience on the use of AIs derives from oncology. Most treatment-related AEs are a direct consequence of estrogen levels suppression. Through negative feedback, AIs induce the release of the gonadotropins, which stimulates the development of follicles in the ovaries that became functional cysts due to absent ovulation; indeed, the administration of AIs for few days have been proposed for follicles recruitment as an alternative to clomiphene citrate [77]. To limit this AE and to maximize activity in breast cancer, bilateral oophorectomy, or ovarian function suppression with GnRH analogs is mandatory during AI treatment [78–81]. The hypoestrogenic state due to AI therapy causes menopause symptoms and loss of BMD [82]. Hot flushes, vaginal dryness, arthralgia, and an increased risk of osteopenia, osteoporosis, and fracture have to be considered before administering these drugs. Before considering their administration and during therapy a periodic BMD follow-up is recommended [77]. Additionally, AIs have been associated with cardiovascular toxicity, alteration of lipid profile, fatigue, forgetfulness, and sleep disturbances [83]. Overall, the pregnant status is an absolute contraindication to AIs [84]. However, additional contraindications should be considered based on the actual indication of AIs and the risk of developing AEs based on dosages and administration. When AIs are adopted in a different context than breast cancer, having the purpose, such as in endometriosis, of ameliorating pain symptoms and quality of life, even osteopenia can be considered an exclusion criterion for the prolonged use of AIs.

Based on their mechanisms of action, the critical role of aromatase in estrogen biosynthesis, and taking into account the experience from breast cancer, AIs have been investigated as a treatment for endometriosis. The first application was the letrozole administration to a postmenopausal woman with aggressive refractory disease in 1998. The patient experienced a dramatic resolution of implants and disease-associated symptoms [85,86]. This systematic review aims to provide the reader a complete overview of available evidence about the adoption of AIs in women affected by endometriosis, summarize what is known, what is unknown, and highlight new lines of research.

3.1 Methods
A systematic literature search was performed from January 1990 to April 2020 in the electronic database MEDLINE, EMBASE, The Cochrane Library, and Web of Science. The search strategy included the combinations of the Medical terms “endometriosis,” “aromatase,” and “aromatase inhibitors.” The literature research aimed to identify all clinical studies published in the English language investigating the application of AIs in patients affected by endometriosis. The following criteria were adopted for considering studies eligible: clinical trials on humans with a minimum of 6 subjects included. They had a diagnosis of endometriosis (any type of diagnosis) and underwent AI administration with or without add-back therapy, after surgery or as exclusive therapy. Case reports, case series, and reports on adenomyosis or endometrial cancer were excluded. Titles and abstracts of identified studies were screened independently by two review authors (S.G., A.S.L.). The full text of the potentially eligible studies was retrieved and independently assessed for eligibility by another two review team members (J.C., F.B.). Any disagreement over the eligibility of particular studies was resolved through discussion with a third author (S.F.). The reference lists of all identified studies were systematically revised to identify other eligible publications.

3.2 Results

From the literature search, 701 records were identified, 234 manuscripts were excluded for duplication, and 53 were selected for the full-text evaluation. Finally, 16 clinical studies were identified, among which 15 were included in the final review. The study which was excluded involved only patients with adenomyosis [87]. We identified eight pilot studies including a single arm of 6 - 20 patients [88–95], three non-randomized open-label controlled clinical trials [96–98], and four randomized controlled clinical trials, of which three open-label [99–101] trials and one double-blind trial [102].

All trials identified tested the use of anastrozole or letrozole; in none of them, exemestane was adopted. Letrozole 2.5 mg/day was the most investigated AI in endometriosis, being used in 11 out of 15 trials included. Eight trials administered letrozole at the dosage of 2.5 mg/day for six months; other two trials administered letrozole 2.5 mg/day for 2 and 12 months, respectively [88,91,92,94,96–101]. One trial was based on letrozole 5mg/day for three months [95], and a second trial on letrozole at 0.25 mg/day by the use of a 2g vaginal suppository [90]. Only three out of 15 trials investigated anastrozole at the dosage of 1 mg/day; in two of them, it was used for six months, and in one for three months [89,93,102]. Most trials
administered AIs supplemented with calcium citrate (1,000-2,400 mg/day) and vitamin D (800-880 UI/day) on daily basis [88,90–92,94,96–99,101,102]. Noteworthy, only three trials administered the AI alone (with or without calcium citrate and vitamin D) [90,99,100]. Eight trials administered AIs combined with norethindrone acetate (NETA); in two of them, AI was combined to a GnRH analog, in the other two to desogestrel and combined oral contraceptive (COC).

The majority of populations included in these studies consisted of premenopausal women with symptoms refractory and persistent or signs of endometriosis after at least one previous line of therapy; usually, AIs were started after a minimum of three months from previous treatment suspension. All studies excluded women with osteopenia or osteoporosis. Diagnosis of endometriosis was surgically done at laparoscopy for all patients included in 9 trials [88,89,91,94,96,99–102]; histologic confirmation both from surgery or biopsy was obtained in 3 studies [90,92,97]; ultrasound was only adopted in 3 trials [93,95,98]. Details of study design, therapeutic regimes, length of follow-up, and main findings of studies included in this systematic review are reported in Table 1.

### 3.2.1 Efficacy

The first trial demonstrating symptoms improvement and disease stage reduction after the administration of AIs was published in 2004 by Ailawadi et al. [88,103]. They performed a single-arm open-label trial, including 10 premenopausal women with endometriosis with refractory or recurrent pelvic pain. All patients underwent diagnostic laparoscopy after three months from medical therapy discontinuation and one month before starting AIs. Letrozole 2.5 mg/day plus NETA 2.5 mg/day were administered for six months. A significant reduction in ASRM score and endometriosis stage was observed at the laparoscopy performed within one month after treatment conclusion (44.1±29.7 versus 5.4±5.64; p=0.0013). Nine out of 10 women had improved symptoms with a significant reduction in pain (mean pain: 6.22±2.07 versus 2.52±2.09; p<0.001) [88]. Remorgida et al. [92] evaluated the same therapy in 12 patients with rectovaginal endometriosis and refractory or recurrent pelvic pain. Dysmenorrhea, dyschezia, CPP, and quality of life (QoL) were significantly improved after six months of treatment. However, pain symptoms and QoL were reported similar to baseline values after six months of follow-up; moreover, 5 out of 12 patients underwent surgery. The third single-arm trial by Ferrero et al. [94] confirmed the efficacy of letrozole combined with
NETA for controlling pain symptoms in 6 patients affected by colorectal endometriosis. Gastrointestinal symptoms, such as diarrhea, cramping and bloating ameliorated, but constipation persisted. Only one out of 4 single-arm trials investigated anastrozole 1 mg/day. Amsterdam et al. [89] enrolled 18 premenopausal women with refractory and recurrent pelvic pain after two therapy lines. At one month from therapy discontinuation, anastrozole in combination with COC was administered for six months; a significant pain reduction (8.24±1.76 versus 4.24±2.70; p<0.001) was observed in 14 out of 18 patients. Although these four trials reported symptoms improvement, the single-arm design and the administration of AIs only combined with NETA or COC did not allow drawing a definitive conclusion on AI efficacy [28–30].

The first controlled trial aimed to investigate the efficacy of AIs in the control of pain symptoms related to endometriosis was published in 2009 by Ferrero et al. [96]; six months of daily treatment with letrozole 2.5 mg plus NETA 2.5 mg were compared with six months of NETA 2.5 mg/day alone. The controlled trial was non-randomized and based on the patient preference; 41 premenopausal women with rectovaginal endometriosis and refractory or recurrent pelvic pain were allocated in each treatment arm after three months from previous treatment interruption. The intensity of dysmenorrhea, dyspareunia, and CPP significantly decreased during treatment in both groups; dyspareunia and CPP resulted in significantly lower scores in the group who received letrozole at three and six months of therapy. However, all patients achieved pain intensity similar to baseline values at six and twelve months of follow-up after the treatment interruption. Moreover, a significantly higher incidence of AEs was reported in the letrozole group, although no difference in patients’ dropout was observed.

The same authors performed a second controlled clinical trial [101], randomizing 35 premenopausal women with deep endometriosis and pain symptoms lasting 12 months or more in two groups. Seventeen patients underwent six months of daily letrozole 2.5 mg plus NETA 2.5 mg, and 18 women received letrozole 2.5 mg daily and triptorelin depot 11.25 mg every three months. Significant reduction of pain symptoms and Biberoglu and Behrman scale score [104] was observed in both treatment groups with no significant differences; conversely, a significantly higher reduction of rectovaginal endometriotic nodules volume was observed in the group who received triptorelin. However, AEs caused the drop out of 8 patients enrolled in the letrozole plus triptorelin group. Regarding the volume reduction of rectovaginal endometriotic nodules, the same group published in 2013 an open-label non-randomized controlled trial in which 8 women who
received letrozole plus NETA were compared with patients who underwent other regimens (NETA, triptorelin, desogestrel, COC). A significant and comparable reduction in rectovaginal nodule volume was observed after six months of therapy in all the treatment options without inter-group differences [97].

All these three controlled trials by Ferrero et al. investigated the combined therapy of daily letrozole 2.5 mg plus NETA 2.5 mg administered for six months. Overall, their results demonstrated an additional effect of letrozole in controlling pain symptoms than NETA alone and no relevant advantages when a GnRH analog was used instead of NETA. Letrozole was associated with a higher incidence of AEs than NETA alone but still tolerated; conversely, letrozole plus GnRH analog caused numerous drops out.

So far, four single-arm studies and three controlled trials investigated AIs (all but one letrozole 2.5 mg/day) administered combined with NETA or COC to treat endometriosis in symptomatic patients. Conversely, Roghaei et al. [99] performed a randomized controlled trial in which six months of letrozole 2.5 mg daily alone was compared to danazol 600 mg/die, and placebo as postoperative treatment. All three arms reported symptoms improvement in the first three postoperative months; a further and persistent reduction of pain symptoms was observed only in the two treatment groups, without differences between letrozole and danazol. Other two trials tested AIs as postoperative treatment: Soysal et al. [102] published in 2004 a randomized, double-blind, controlled trial, in which 80 patients with severe endometriosis (rASRM score > 40) were randomized after surgery to six months of anastrozole 1 mg/day plus goserelin every four weeks versus six months of goserelin alone. The trial aimed to investigate whether the combined therapy could lower the recurrence risk and extend the symptom-free interval. After 24 months of follow-up, the median time to detect symptoms recurrence was significantly longer for the anastrozole group (>24 months versus 17 months; p=0.0089); 3 (7.5%) out of 40 patients reported symptoms recurrence in the goserelin plus anastrozole group as compared to 14 women (35%) in the goserelin only group. No differences were observed in the scales and indexes used to assess the menopausal QoL. The third trial was conducted by Alborzi et al. [100] to evaluate whether two months of medical therapy administered after surgical treatment can improve pain symptoms and reproductive outcomes. Infertile women diagnosed with endometriosis confirmed during laparoscopic surgery were randomized to three postoperative managements after excluding severe male factor. Fifty-eight patients received two months of letrozole 2.5 mg/day, 58 women received triptorelin 3.75 mg every four weeks, and 59 patients did not receive treatment. After a minimum of one year
of follow-up, no differences were observed in endometriosis recurrences, pain symptoms, and reproductive outcomes; only a higher significant prevalence of functional cysts was observed in the letrozole group (24.3% versus 2.5%; \( P<0.001 \)). Two of these three trials investigate letrozole 2.5 mg/day alone as postoperative maintenance treatment without reporting differences with compared groups. Conversely, Soysal et al. [102] demonstrated a reduced risk of recurrence when the six-month postoperative therapy was conducted with anastrozole 1 mg/day added to the GnRH analog.

Three trials investigated as the primary outcome the effect of AIs on the size of endometriomas; two single-arm open-label clinical trials [93,95], and one control open-label trial [98]. Lossl et al. [93] enrolled 20 women with the ultrasonographic diagnosis of endometrioma > 20 mm and planned assisted reproductive technique. Anastrozole 1 mg/day and goserelin every four weeks were administered for three months, followed by controlled ovarian stimulation and in vitro fertilization. The endometrioma volume was reported reduced, stable, and increased (>25%) in 75%, 10%, and 15% of women, respectively; a concomitant reduction in Ca-125 serum levels was observed. The second single-arm study was published in 2015 by Agarwal et al. [95]; 8 premenopausal women with a total of 14 endometriomas with diameter > 30 mm at ultrasound evaluation were enrolled, and letrozole 5 mg plus NETA 5 mg were administered for three months. Mean endometrioma diameter and volume significantly decreased by 50% (4.6 ± 1.6 cm versus 2.3 ± 1.6 cm; \( P<0.01 \)) and 75% (60.1 ± 58.7 cm\(^3\) versus 15.0 ± 16.4 cm\(^3\); \( P<0.01 \)), respectively; significant reduction in symptoms (dyspareunia and CPP) and Biberoglu and Behrman scale score were reported. These results were confirmed in the open-label non-randomized control trial by Ferrero et al. [98]. They enrolled 40 premenopausal women with unilateral endometrioma stable after two months of follow-up after six months of washout from any hormonal therapy. Patients were allocated 1:1 to receive daily letrozole 2.5 mg plus NETA 2.5 mg or NETA 2.5 mg/die alone for six months. Significant reduction in mean endometrioma volume was observed after three and six months of treatment in both study groups; after six months of therapy, the volume reduction was significantly higher in the group of patients who received the combined therapy (-74.4 ± 4.2% versus -46.8 ± 3.8%; \( p < 0.001 \)). Both groups reported a significant reduction in dysmenorrhea, dyspareunia, and CPP at the end of therapy, with a comparable proportion of patients satisfied by the treatment. No significant differences were observed about AEs. The three trials that focused on endometriomas adopted AIs with three different protocols. Among these, the most important is a controlled
trial that investigated the combined therapy of daily letrozole 2.5 mg plus NETA 2.5 mg administered for six months. The results demonstrated an improved effect on endometriomas provided by the addition of AIs. However, the volume of endometrioma and symptoms returned to baseline values in both groups after six months from therapy discontinuation.

3.2.2 Adverse effects

The development of functional cysts was not uncommon in trials related to AIs for the treatment of endometriosis. In the trial conducted by Alborzi et al. [100], 24.3% of women who received two months of letrozole 2 mg/day alone developed functional cysts as compared to 2.5% in the triptorelin group (P<0.001). The same effect was observed in the single-arm open-label trial by Remorgida et al. [91], which aimed to evaluate the efficacy of letrozole in the treatment of refractory or recurrent pelvic pain. Twelve premenopausal women with regular menses and IV stage endometriosis received letrozole 2.5 mg/day plus desogestrel 75 μg/day for six months. All patients interrupted the treatment due to the development of functional ovarian cysts. Multiple ovarian cysts were developed by 66.7% of patients, with the largest average diameter of 5.0 ± 1.3 cm. This AE was not reported by Roghaei et al. [99] after six months of letrozole 2.5 mg/day as monotherapy. However, the absent use of ultrasound during the follow-up may explain the missed detection of ovarian cysts, nevertheless suggesting that their development may not have clinical implications. In any case, this AE is one of the main reasons to adopt a combined therapy during the administration of AIs for endometriosis. The association of medication suppressing the release of gonadotropins, such as NETA or GnRH analogs may prevent the formation of functional cysts, as shown by all the other trials adopting a combined therapy.

Regarding the loss of BMD [82], all trials on endometriosis excluded women with osteopenia; however, in 11 out of 15 trials AIs were administered in combination with elemental calcium and vitamin D as a preventive strategy [88,90–92,94,96–99,101,102]. Moreover, BMD was assessed before and after treatment in most cases. A significant reduction of BMD was observed at the end of therapy and after 24 months of treatment compared to baseline in both treatment groups of the randomized controlled trial by Soysal et al. [102]. Noteworthy, AI plus GnRH analog caused significantly higher BMD loss than GnRH analog alone at the end of therapy but not after 24 months of follow-up. However, in all single-arm trials, BMD loss was not
observed; the administration of AIs combined with NETA or COC in these studies may explain this finding [88,89,92,94]. The induced BMD reduction using AIs in endometriosis was confirmed by Ferrero et al., who concomitantly administered letrozole 2.5 mg/day with triptorelin 11.25 mg every three months [101]. The 10 women who completed the six months of treatment reported a significant reduction in BMD at the end of treatment. Conversely, women randomized to six months of letrozole 2.5 mg/day plus NETA 2.5 mg/day did not experience significant BMD changes. The two other control trials by the same group confirmed the preventive effect exerted by the add-back therapy with progestins and estroprogestins [96,98], without difference between letrozole plus NETA and NETA alone. Therefore, add-back treatment with NETA or COC has been proposed as the first option for using AIs in women with endometriosis to prevent the development of functional cysts and loss of BMD [40,101].

The administration of AIs to treat endometriosis has been associated with the development of menopausal symptoms [100,102]. Different trials reported hot flushes, hair loss, and vaginal dryness, which were reduced when AIs were administered combined with NETA or COC. In the study by Ferrero et al. [101], women who received letrozole plus triptorelin reported a higher rate of AEs than the group who received letrozole plus NETA (77.8% versus 35.3%). Moreover, hot flushes, hair loss, and vaginal dryness were reported only in the first group. However, the combination of NETA with AIs did not prevent experiencing AEs as compared to NETA alone. In both trials by Ferrero et al. [96,98], in which letrozole plus NETA was compared to NETA alone, a higher proportion of patients in the group who received also letrozole reported at least one AE (35% versus 55%; p = 0.340 and 18.4% versus 43.2%; p = 0.020). Irregular bleeding, depression, weight gain, insomnia, migraine, and decrease of libido were reported in both treatment groups [96,98]. Conversely, myalgia, arthralgia, fatigue, hair loss, vaginal dryness, and hot flashes were more specifically reported with the administration of the AI.

Concerning cardiovascular AEs and lipid profile, none of the trials on AIs for endometriosis reported significant toxicity on blood count, lipid profile, or liver and renal function.

### 3.2.3 Innovation and early clinical development

Innovation in pharmacotherapy includes the development of new molecules and delivery methods for improving the efficacy of the drug as well as reducing treatment-related AEs [31].
The vaginal route is a well-known administration route that can reduce therapeutic dosages of hormones, maintaining efficacy with a better safety profile [105]. Moreover, this route is of particular interest for patients with rectovaginal endometriosis, who could take advantage of the local administration. In this regard, Hefler et al. [90] conducted a single-arm open-label clinical trial in which 10 premenopausal women with rectovaginal endometriosis were treated for six months with daily anastrozole 0.25 mg administered in a 2-g vaginal suppository. They established dosages based on the higher resorption of vaginal walls and data from danazol suppositories employed to treat endometriosis; all patients received supplementation with elemental calcium 2,400 mg and vitamin D 800 IE daily.

Nevertheless, this administration route did not provide similar results of other studies; as compared to baseline values, CPP and dyspareunia did not improve during and after the therapy. Only dysmenorrhea and QoL assessed with the SF-36 showed a significant improvement. No changes in gonadotropin levels, estradiol levels, and BMD were reported.

These mixed results did not decrease the interest in the vaginal administration of AIs for endometriosis treatment. Schultze-Mosgau et al. [106] conducted a randomized, three-arm, parallel-group, open-label, phase I trial to assess the pharmacokinetics, pharmacodynamics, and safety of three-dose combinations of anastrozole and levonorgestrel administered by a vaginal ring. Sixty healthy women aged 18-35 years were randomized to the three different drug doses, given by vaginal ring for two consecutive 28-day periods. This study allowed defining the anastrozole and levonorgestrel doses to be investigated to treat endometriosis in phase II trials [107]. A randomized, parallel-group, double-blind phase IIb clinical trial has been recently concluded based on these results. The study evaluated the efficacy and safety of levonorgestrel 40 mcg with or without different doses of anastrozole administered by vaginal ring for three months compared to placebo and leuprolide (NCT02203331). The results will allow us to define whether the adoption of a vaginal ring may represent an innovation in the use of AIs to treat endometriosis.

4. Conclusion

Evidence from the current literature on advantages provided by the use of AIs for the treatment of endometriosis is preliminary and limited. Data from eight single-arm trials have been not conclusive. Regarding the seven controlled trials, four trials by Ferrero et al. investigated the combined therapy of daily
letrozole 2.5 mg plus NETA 2.5 mg for six months. Overall, they demonstrated the superiority of the combined treatment in controlling pain symptoms and causing a reduction of endometrioma volume compared to NETA alone [96,98]. However, letrozole has been associated with a higher incidence of AEs than NETA alone.

Among the other three controlled trials, only Soysal et al. [102] reported a reduced risk of endometriosis recurrence, observing an extended symptom-free interval after conservative surgery when the GnRH analog was combined with anastrozole 1 mg/day. Nevertheless, they reported higher BMD loss in patients receiving the combined treatment.

The presence of only three trials supporting the efficacy of AIs in endometriosis highlights the need for further evidence; therefore, AIs should be employed in selected patients as a second-line option in the research setting. Moreover, the frequency and severity of AEs during AI therapy need to be better characterized. In this regard, available data suggest administering AIs in combination with add-back therapy (NETA or COC) to reduce treatment-related AEs. However, the most appropriate add-back therapy, as well as the supplementation of calcium citrate and vitamin D, is not defined yet. Further research should aim to determine the best dosages, treatment length, appropriate add-back therapies, innovative administration ways of AIs, and characterize which patient may benefit more from their administration.

5. Expert Opinion

Estrogens have a critical role in the development, support, and progression of endometriosis [24]; indeed, most therapeutic options aim to inhibit the production and action of estrogens. Directly targeting endometriotic implants blocking the local production of estrogens [108], AIs are an attractive therapeutic option in women affected by endometriosis [73]. Being aromatase a rate-limiting step in all tissues able to produce estrogen, the block of this enzyme can provide a more marked suppression of estrogen levels compared to other drugs inhibiting the ovarian function [40]. AIs may represent an alternative therapeutic option in patients who have intermittent or limited benefit with progestins, estroprogestins, and GnRH analogs [11].

Based on the available evidence, further research is needed to clarify the efficacy and clinical utility of AIs for the treatment of endometriosis. Moreover, the preventive role of add-back therapy needs to be addressed.
on that basis, AIs should be adopted only as second-line therapy in women who are refractory to the standard treatments [11,77].

Recently, the presence of aromatase in endometriotic implants has been questioned by some studies that did not confirm the presence of the enzyme [73]. Nevertheless, assuming that aromatase may be variably present in the endometriotic implants of patients affected is questionable. On that basis, other research lines should be focused to characterize better the patients affected by endometriosis; moreover, it could be of interest to identify in which women AIs can have a higher therapeutic activity; this may be theoretically done by identifying aromatase in endometriotic implants after surgery. In these patients, the use of AIs combined with NETA might represent the best option to reduce the risk of recurrence and length of the symptom-free interval after surgical treatment; conversely, in patients without aromatase, alternative medical therapies could be employed. Similarly, identifying women in which peripheral tissues represent the primary source of estrogen means identifying those patients in which AIs may have significant therapeutic effects, such as obese and postmenopausal women [109].

Future research lines should also be focused on different points still undefined about the administration of AIs for endometriosis, such as the best therapeutic dosage, the most appropriate add-back therapy, the best way of administration, and the proper length of treatment. The most investigated regimen, which was demonstrated effective, is letrozole at the dosage of 2.5 mg/day combined with NETA 2.5 mg/day for six months; however, multiple regimens have been proposed, and none of the studies have compared them until now. Moreover, both the therapeutic role and the preventive role of AIs after surgery, especially after the appropriate characterization of the disease from pathological investigations, have to be characterized. AIs may represent a therapeutic option in endometriosis for patients with symptomatic disease refractory to other treatments. Future studies will draw a definitive conclusion on the role and future perspective of AIs administration for treating endometriosis.

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* Overview of current and investigational drugs for endometriosis


* Accurate and complete review on the etiopathogenesis of endometriosis


*Overview of current and novel drug delivery methods for endometriosis*


*Manuscript about the pathogenetic role of aromatase in endometriosis*


*Manuscript presenting the role of aromatase in gynecological diseases including endometriosis*


Table 1. Clinical trials that investigated the adoption of aromatase inhibitors in women with endometriosis.
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<table>
<thead>
<tr>
<th>Authors</th>
<th>year</th>
<th>Design</th>
<th>Patient number</th>
<th>Treatments</th>
<th>TPL (m)</th>
<th>FU (m)</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ailawadi et al.</td>
<td>2004</td>
<td>single arm</td>
<td>10</td>
<td>• Letrozole 2.5mg/d + NETA 2.5mg/d + Ca 1,250mg/d + and vitamin D 800 IU/d</td>
<td>6</td>
<td>-</td>
<td>Improved ASRM score and symptoms, stable BMD</td>
</tr>
<tr>
<td>Soysal et al.</td>
<td>2004</td>
<td>double arm, DB, RND</td>
<td>40 vs 40</td>
<td>• Anastrozole 1mg/d + goserelin 3.6mg/4w + Ca 1200mg/d + vitamin D 800 IU/d</td>
<td>6</td>
<td>24</td>
<td>In anastrozole group higher median time to recurrence after surgery (&gt;24 m vs 17 m; p=0.0089); recurrence in 3/40 vs 14/40 after 24 m; higher BMD loss after 6 m.</td>
</tr>
<tr>
<td>Amsterdam et al.</td>
<td>2005</td>
<td>single arm</td>
<td>18</td>
<td>• Anastrozole 1mg/day + ethinyl estradiol 20 mcg/levonorgestrel 0.1mg/day</td>
<td>6</td>
<td>-</td>
<td>Improved pain symptoms, stable BMD</td>
</tr>
<tr>
<td>Hefler et al.</td>
<td>2005</td>
<td>single arm</td>
<td>10</td>
<td>• Letrozole (0.25 mg) in 2g vaginal suppository + Ca 2400mg/d + vitamin D 800 IU/d</td>
<td>6</td>
<td>-</td>
<td>Improved dysmenorrhea and QoL, stable dyspareunia and CPP, stable BMD</td>
</tr>
<tr>
<td>Remorgida et al.</td>
<td>2007</td>
<td>single arm</td>
<td>12</td>
<td>• Letrozole 2.5mg/d + NETA 2.5mg/d + Ca 1000mg/d + vitamin D 880 IU/d</td>
<td>6</td>
<td>12</td>
<td>Improved pain symptoms and QoL, stable BMD</td>
</tr>
<tr>
<td>Remorgida et al.</td>
<td>2007</td>
<td>single arm</td>
<td>12</td>
<td>• Letrozole 2.5mg/d + desogestrel 75mcg/d + Ca 1000mg/d + vitamin D 880 IU/d</td>
<td>6</td>
<td>6</td>
<td>Interrupted for functional ovarian cysts</td>
</tr>
<tr>
<td>Ferrero et al.</td>
<td>2009</td>
<td>double arm, OL, non-RND</td>
<td>41 vs 41</td>
<td>• Letrozole 2.5mg/d + NETA 2.5mg/d + Ca 1000mg/d + vitamin D 880 IU/d</td>
<td>6</td>
<td>12</td>
<td>Comparable pain symptoms and QoL improvement; Lower dyspareunia and CPP after 3 and 6 m with letrozole, stable and comparable BMD</td>
</tr>
<tr>
<td>Study Ref.</td>
<td>Year</td>
<td>Study Design</td>
<td>Treatment 1</td>
<td>Treatment 2</td>
<td>Treatment 3</td>
<td>N1</td>
<td>N2</td>
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<tr>
<td>Lossl et al. [93]</td>
<td>2009</td>
<td>Single arm</td>
<td>Anastrozole 1 mg/d + goserelin 3.6 mg/4w + subsequent COS and ART</td>
<td></td>
<td></td>
<td>3</td>
<td></td>
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<tr>
<td>Ferrero et al. [94]</td>
<td>2010</td>
<td>Single arm</td>
<td>Letrozole 2.5 mg/d + NETA 2.5 mg/d + Ca 1000 mg/d + vitamin D 880 IU/d</td>
<td></td>
<td></td>
<td>6</td>
<td>6</td>
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<tr>
<td>Roghaei et al. [99]</td>
<td>2010</td>
<td>Triple arm, OL, RND</td>
<td>Letrozole 2.5 mg/d + Ca 1000 mg/d + vitamin D 800 IU/d</td>
<td>Danazol 600 mg/d + Ca 1000 mg/d + vitamin D 800 IU/d</td>
<td>Ca 1000 mg/d + vitamin D 800 IU/d</td>
<td>38 vs 37 vs 31</td>
<td>6</td>
</tr>
<tr>
<td>Alborzi et al. [100]</td>
<td>2011</td>
<td>Triple arm, OL, RND</td>
<td>Letrozole 2.5 mg/d</td>
<td>Triptorelin 3.75 mg/4w</td>
<td>Control</td>
<td>47 vs 40 vs 57</td>
<td>2</td>
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<tr>
<td>Ferrero et al. [101]</td>
<td>2011</td>
<td>Double arm, OL, RND</td>
<td>Letrozole 2.5 mg/d + NETA 2.5 mg/d + Ca 1000 mg/d + vitamin D 880 IU/d</td>
<td>Triptorelin 11.25 mg/3m + Ca 1000 mg/d + vitamin D 880 IU/d</td>
<td></td>
<td>17 vs 18</td>
<td>6</td>
</tr>
<tr>
<td>Ferrero et al. [97]</td>
<td>2013</td>
<td>Multiple arm, OL, non-RND</td>
<td>Letrozole 2.5 mg/d + NETA 2.5 mg/d + Ca 1000 mg/d + vitamin D 880 IU/d</td>
<td>NETA, or triptorelin + tibolone, or desogestrel, or sequential oral contraceptive pill</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Year</td>
<td>Design</td>
<td>N</td>
<td>Treatment</td>
<td>Follow-Up</td>
<td>Results</td>
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<tr>
<td>Ferrero et al. [98]</td>
<td>2014</td>
<td>double arm, OL, non-RND</td>
<td>20 vs 20</td>
<td>Letrozole 2.5mg/d + NETA 2.5mg/d + Ca 1000mg/d + vitamin D 880 IU/d + NETA 2.5 mg/d</td>
<td>6 vs 6</td>
<td>Comparable pain symptoms improvement, higher reduction in endometrioma volume in letrozole group (-74.4±4.2% vs -46.8±3.8%). Return to baseline in both group after 6 months of follow-up; stable and comparable BMD</td>
<td></td>
</tr>
<tr>
<td>Agarwal et al. [95]</td>
<td>2015</td>
<td>single arm</td>
<td>8</td>
<td>Letrozole 5mg/d + NETA 5mg/d</td>
<td>3</td>
<td>Pain symptoms improvement and reduction in endometrioma volume: -50% diameter and -75% in volume</td>
<td></td>
</tr>
</tbody>
</table>

OL, open label; RND, randomization; TPL, therapy length; FU, follow-up; d, day; w, week; m, months; NETA, Norethisterone acetate; Ca, calcium; ASRM, America society of reproductive medicine; BMD, bone mineral density; QoL, Quality of life; CPP, chronic pelvic pain; RV, rectovaginal; vs, versus.