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This is the peer reviewed version of the following article:

*Original:*

Tosti, C., Biscione, A., Morgante, G., Bifulco, G., Luisi, S., Petraglia, F. (2017). Hormonal therapy for endometriosis: from molecular research to bedside. EUROPEAN JOURNAL OF OBSTETRICS, GYNECOLOGY, AND REPRODUCTIVE BIOLOGY, 209, 61-66 [10.1016/j.ejogrb.2016.05.032].

*Availability:*

This version is available <http://hdl.handle.net/11365/998396> since 2016-11-08T12:14:38Z

*Published:*

DOI: <http://doi.org/10.1016/j.ejogrb.2016.05.032>

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# Hormonal therapy for endometriosis: from molecular research to bedside

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## ABSTRACT

Endometriotic lesions are associated with hormonal imbalances, including increased oestrogen synthesis and metabolism, and progesterone resistance. These hormonal changes are associated with increased proliferation, inflammation, pain and infertility. Hormone imbalances are targets for treatment, and therapeutic strategies and innovations of hormonal drugs for endometriosis are increasing. Hormonal drugs that decrease systemic and local oestrogen synthesis [gonadotrophin-releasing hormone (GnRH) analogues, GnRH antagonists, aromatase inhibitors] or oestrogen activity (selective oestrogen receptor modulators) act on oestrogen receptors. Progesterone resistance is counteracted by progestins (medroxyprogesterone acetate, dienogest, danazol, levonorgestrel) and selective progesterone receptor modulators (a class of drugs under development). As endometriosis is a chronic disease, there is a need to develop drugs that can be used in the long term and have few side-effects.

*Keywords:*

Endometriosis

Hormonal treatment

Pathogenesis

Oestrogen secretion

Progesterone receptor activity

**Condensation**

Endometriotic lesions are associated with hormonal imbalances, including increased oestrogen synthesis and metabolism, and progesterone resistance. Therapeutic strategies and innovations of hormonal drugs for endometriosis are increasing. As endometriosis is a chronic disease, there is a need to develop drugs that can be used in the long term and have few side-effects.

Accepted Manuscript

## 1. Introduction

The pathogenesis of endometriosis involves several mechanisms, including cell proliferation and differentiation, apoptosis, migration, adhesion and invasion, inflammation and neuroangiogenesis. The main basic event is well recognized and consists of altered expression of oestrogen (ERs) and progesterone (PRs) receptors in endometriotic tissue; in addition, biologically significant quantities of sex steroid hormones are produced in endometriotic tissue, thus influencing the cascade of endometriotic pathogenetic mechanisms [1]. Therefore, at present, the most common drugs used for the treatment of endometriosis target ERs or PRs.

## 2. Increased ER sensitivity

Endometriosis is considered to be an oestrogen-dependent disease, in which increased ER activity is a major contributing factor to cell proliferation and disease progression [1,2]. Endometriotic lesions show high oestradiol (E2) biosynthesis and low E2 inactivation compared with healthy women [3]. Several enzymes that metabolize oestrogen (aromatase,  $17\beta$ -hydroxysteroid dehydrogenase type 2- $17\beta$ -HSD2) are expressed aberrantly in ectopic endometrium, which can lead to high E2 biosynthesis and local E2 inactivation; subsequently, excess local E2 results in further proliferation of the lesions. ER binding to oestrogen-responsive elements in the promoters of their target genes and ER $\beta$  expression are highly upregulated due to decreased promoter methylation [4]. ER $\beta$  suppresses ER $\alpha$  expression, yielding an augmented ER $\beta$ /ER $\alpha$  ratio, which may cause a shift from E2 stimulation to E2 inhibition of PR expression [5].

Gonadotrophin-releasing hormone (GnRH) analogues act on oestrogen secretion and/or ER activity, and GnRH antagonists, aromatase inhibitors and selective oestrogen receptor modulators (SERMs) are in development.

### 2.1. GnRH analogues

Goserelin, leuprolide, nafarelin, buserelin and triptorelin are effective for the treatment of endometriosis-related pain [6]. GnRH analogues are effective for pain control, and have been considered to be the gold standard for two decades. GnRH analogues are currently considered as second-line therapy, when first-line treatments fail, are not tolerated or are contraindicated [7].

Treatment with GnRH analogues has some limitations, including the high recurrence rate (50% of patients show a relapse of symptoms within 6 months of discontinuation of therapy) and side-effects associated with the transitory pharmacological menopause condition created (i.e. loss of bone density, worsening of serum lipoprotein cholesterol distribution, hot flushes, genitourinary atrophy, depression and decreased libido) [8]. It is necessary to monitor bone density during treatment. However, the prolonged induced hypogonadism is reversible and may be minimized by an add-back therapy (exogenous administration of sex steroids). With this approach, the administration of GnRH analogues, which was initially limited to 6 months, could be prolonged to up to 2 years; common regimens are low-dose combined oestroprogestins, oestrogen or progestins alone, bisphosphonates, tibolone or raloxifene [9] (Fig. 1).

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## 2.2. GnRH antagonists

GnRH antagonists act by binding to the same pituitary GnRH receptors as GnRH analogues, and result in immediate suppression of the production and release of gonadotropins and gonadal steroids [2]. A study on patients with endometriosis found that subcutaneous cetrorelix reduced symptoms and improved the stage of disease (from stage III to stage II). Few side-effects were reported, such as irregular genital bleeding in 20% of patients [10]. An oral, non-peptide GnRH antagonist, elagolix, caused dose-related pituitary and gonadal suppression in healthy premenopausal women within 24 h in subjects receiving a dose of  $\geq 50$  mg/day [11]. Its safety and efficacy in the treatment of endometriosis-associated pain was subsequently evaluated in a phase 2 randomized trial in 155

women with laparoscopically confirmed endometriosis [12]. Elagolix demonstrated an acceptable efficacy and safety profile in women with endometriosis-associated pain, with minimal bone mineral density changes during treatment. It is currently unclear what constitutes a safe duration of treatment with a GnRH antagonist [13]. This class of medication may become a valuable tool in the treatment of endometriosis, and further studies are underway (Fig. 1).

### 2.3. Aromatase inhibitors

Aromatase inhibitors impair the conversion of androgens to oestrogens by inhibiting aromatase activity, and suppress ovarian and local oestrogen production in endometriotic tissue [14]. The third-generation aromatase inhibitors (anastrozole, letrozole, exemestane and vorozole) are more potent and specific for the aromatase enzyme, and are associated with a few side-effects such as headache, nausea and diarrhoea [15]. However, eutopic endometrial cell cultures exposed to anastrozole and letrozole consistently demonstrate that, contrary to what might be expected, cell proliferation is enhanced [16,17].

The use of letrozole in a murine model of endometriosis demonstrated significantly smaller lesions [18], significantly decreased VEGF immunoreactivity and prostaglandin E levels, and increased apoptosis [19,20,21].

A number of non-randomized studies conducted in patients with endometriosis treated with aromatase inhibitors (either as single therapy or in combination) have been published [11,22,23]. Patients with chronic pelvic pain refractory to conventional therapies, and vaginal or bowel endometriosis were included. The number of patients varied between four and 16 in each series, and letrozole or anastrozole was used. The aromatase inhibitors were used alone or combined with progestogens, contraceptive pills, calcium and/or vitamin D, and therapy lasted predominantly for 6 months. Pain reduction was reported in all series, with a clear effect on dysmenorrhoea, dyspareunia, and physical and social functioning. A global pattern of recurrence of pain was observed after the termination of treatment. The reported side-effects were irregular bleeding,

weight gain and joint pain.

A prospective trial compared the effects of letrozole plus norethisterone acetate with norethisterone acetate alone for 6 months in women with rectovaginal endometriosis-associated pain, and found that the women who received letrozole plus norethisterone acetate had significantly lower pain intensity and dyspareunia. However, these women experienced more side-effects, particularly joint pain and myalgia [24].

A randomized, double-blind study compared 6 months of goserelin plus anastrozole with goserelin alone as postoperative therapy for severe endometriosis. A significantly longer time to symptom recurrence was observed in women who received the combined regimen, and no differences in quality of life or bone mass were found between the groups [25].

In another prospective randomized study, letrozole plus norethisterone acetate was compared with letrozole plus triptorelin [26]. Both groups of patients showed a similar reduction in pain symptoms during treatment. The GnRH agonist group experienced a greater reduction in endometriotic nodules, but also experienced arthralgia, decreased libido, hot flushes and depression. Bone mineral density decreased significantly in the GnRH agonist group (Fig. 1).

#### *2.4. Selective oestrogen receptor modulators*

The tissue-selective activity of SERMs qualifies some molecules that are predominantly antagonistic in breast and uterus and agonistic in skeleton. Raloxifene was approved for the management of postmenopausal osteoporosis [27] and does not stimulate endometrial proliferation in rats.

Daily oral administration of raloxifene to rats with induced endometriosis significantly decreased the volume of implants after 14 days of treatment [28]. Bazedoxifene, another SERM that was demonstrated not to stimulate the endometrium in postmenopausal women or to antagonize conjugated oestrogen-induced uterine stimulation, was also found to decrease the mean size of endometriosis-like lesions in rodents. In a double-blind prospective study, patients with

endometriosis-related pelvic pain after surgical treatment were allocated at random to daily raloxifene or placebo for 6 months. The study was halted early because the raloxifene group experienced pain significantly earlier and required re-operation [29]. Therefore, the future trend will be to find SERMs that act as ER antagonists in the modulation of lesions and chronic pelvic pain. Indeed, an experimental study recently revealed the effects of two ER ligands: chloroindazole (CLI) and oxabicycloheptene sulfonate (OBHS). They showed ER-dependent anti-inflammatory activity in a model of endometriosis in mice and in human endometriotic stromal cells in culture. CLI and OBHS prevented lesion expansion and also elicited regression of established lesions; suppressed inflammation, angiogenesis and neurogenesis in the lesions; and interrupted crosstalk between lesion cells and infiltrating macrophages, opening new therapeutic perspectives [30]. In a murine model, the same group studied the beneficial role of a repressor of ER activity (prohibitin 2) in suppressing the progression of endometriosis, mainly acting as a brake on the E2–ER axis [31] (Fig. 1).

### **3. Progesterone resistance**

In addition to oestrogen dependence, several studies have supported a profile of progesterone resistance in the pathogenesis of endometriosis. Endometriotic lesions exhibit an overall reduction in PR expression relative to eutopic endometrium and an absence of PR-B. Additionally, endometrial expression profiling has documented dysregulation of progesterone-responsive genes in the luteal phase [3,5]. PR-B is the main PR involved in this process in the endometrium, as its levels are tightly regulated by E2 during the human menstrual cycle. In patients with endometriosis, transcripts for both PR-A and PR-B are dysregulated in eutopic endometrium, whereas in endometriotic implants, the PR-A transcript alone is present [32]. Progesterone counteracts the effects of E2, in part by stimulating 17 $\beta$ -HSD2 transcription; however, local endogenous E2 synthesis blocks this effect in endometriotic lesions, and progesterone resistance due to abolished PR-B expression is believed to be a contributing pathogenic mechanism in endometriosis, where

progesterone does not trigger the expression of 17 $\beta$ -HSD2 and subsequent metabolism of oestrogen [2,3].

Drugs available that act on progesterone secretion and/or PR activity are the progestins (oral, intravaginal, intrauterine and subcutaneous) and selective progesterone receptor modulators (SPRMs).

### *3.1. Progestins (oral/intravaginal/intrauterine/subcutaneous)*

Oral progestins induce decidualization of both eutopic and ectopic endometrium, with the subsequent possible atrophy of lesions.

#### *3.1.1. Medroxyprogesterone acetate*

Medroxyprogesterone acetate (MPA) is a 17-hydroxy-derived progestin with moderate androgenic activity and minor effects on lipoprotein metabolism. Oral MPA is used for long-term treatment of endometriosis with the same effectiveness as GnRH analogues for reducing pain and improving health-related quality of life [33]. The optimal dose of MPA has not yet been defined, and its main side-effects are localized pain, acne and vasodilatation.

The depot formulation of MPA (DMPA) is used for contraceptive purposes and is widely used in women worldwide. The most common mode of administration consists of a single 150-mg intramuscular injection every 3 months. This progestin was compared with oral low-dose danazol, and showed good results in terms of women's satisfaction and reduction of pain symptoms, although the incidence of side-effects was higher in the DMPA group [34]. DMPA 104 mg has also been studied in subcutaneous formulation as a treatment for endometriosis. In comparison with leuprolide acetate 11.25 mg, it was statistically equivalent for the reduction of pain symptoms. DMPA is associated with fewer hypo-oestrogenic symptoms but more irregular bleeding. DMPA, therefore, is an effective alternative for the treatment for endometriosis, particularly in the USA [35,36] (Fig. 1).

### 3.1.2. Dienogest

Dienogest (DNG) is structurally related to the norethindrone family of testosterone derivatives, but differs from norethisterone acetate by having a cyanomethyl group instead of an ethinyl group at C-17 and by the addition of a double bond between C-9 and C-10 [37]. DNG 2 mg/day induces anovulation moderately, thus suppressing oestrogen production [38]. It also downregulates prostaglandin E<sub>2</sub>, inflammatory cytokines [including interleukin (IL)-6, IL-8 and monocyte chemoattractant protein-1], oestrogen synthetase aromatase and neuroangiogenesis factors [such as vascular endothelial growth factor (VEGF) and nerve growth factor] in endometrial and/or endometriotic cells [39,40,41]. In addition, DNG improves progesterone resistance in endometriotic tissue by increasing the relative expression of PR-B and PR-A, and decreasing the relative expression of ER $\beta$  and ER $\alpha$  [42].

The optimal dose of DNG for the treatment of endometriosis was assessed by two independent clinical studies in Europe and Japan, which included dose range, placebo-controlled, active comparator-controlled and long-term studies [43,44]. It has been proven that a 1-mg dose is not suitable for the high rate of bleeding, and DNG 2 mg once daily was recommended as the optimal dosage for the treatment of endometriosis (also compared with 4 mg) because it improves endometriosis-associated symptoms such as dysmenorrhoea, premenstrual pain and diffuse pelvic pain, and because the percentage of irregular bleeding is statistically lower, confirmed by another study, its overall efficacy, tolerability and safety [45]. Another double-blind, placebo-controlled study on women with stage I–IV endometriosis established that DNG 2 mg once daily was significantly more effective than placebo at reducing endometriosis-associated pelvic pain, with low rates of treatment-related adverse events and similar incidences of study withdrawals [46].

DNG showed similar results to intranasal buserelin acetate in terms of improving endometriosis-associated symptoms, with significantly lower reduction in bone mineral density, fewer hot flushes, but more frequent irregular genital bleeding [47]. Equivalent efficacy to depot

leuprolide acetate in the treatment of endometriosis-associated pain was also shown, combined with advantages in terms of safety and tolerability, including stable oestrogen levels and low incidence of potentially hypoestrogenic effects. Quality-of-life analyses also demonstrated an improvement in both treatment groups, with a slightly greater benefit in the DNG group [48].

Long-term data are available from Japanese and European studies. The Japanese data suggested that the efficacy of DNG increased cumulatively, whereas the long-term effect of DNG on bone mineral density was slight and non-cumulative [49]. The European multicentre trial showed that long-term treatment with DNG had a favourable efficacy and safety profile, with progressive decreases in pain and bleeding irregularities during continued treatment; the decrease in pelvic pain persisted for at least 6 months after treatment cessation [50].

In patients with deep endometriosis (rectosigmoidal and bladder endometriosis), DNG had positive results in terms of reduction of the size of lesions (after 10/11 months of use) and immediate relief of subjective symptoms [51]. As with other progestins, bleeding was common in the trials of DNG treatment. However, most cases of bleeding during DNG treatment were spotting or breakthrough bleeding, which decreased with continued treatment and resolved either during or shortly after the end of treatment [52] (Fig. 1).

### 3.1.3. Danazol

The synthetic androgen 2,3-isoxazol, a derivative of  $17\alpha$ -ethynyl testosterone, has mild androgenic but strong anti-oestrogenic activity. It induces the inhibition of gonadotropin release, determines the competitive inhibition of steroidogenic enzymes, modulates immunological function, and suppresses cell proliferation and clinically reduced endometriosis-associated pain symptoms [53].

Poor tolerability represents the major drawback of oral danazol as a treatment for endometriosis; this agent has both androgenic and anabolic properties, leading to side-effects such as weight gain, acne, seborrhoea, oily hair, headache, changes in serum lipoprotein cholesterol distribution and liver function, vaginal atrophy, endometrial changes, and interference with

regularity of the menstrual cycle. The side-effects of oral danazol led to the search for other routes of administration.

Danazol-loaded intrauterine systems [54] and danazol-loaded vaginal rings [55] significantly decrease dysmenorrhoea, dyspareunia and chronic pelvic pain in women with deeply infiltrating and rectovaginal endometriosis nodules are reduced [56]. Locally administered danazol is associated with lower serum concentrations than oral danazol. Systemic and gynaecological side-effects are seldom observed, and lipid parameters and liver function are reported to be unaltered. A prospective study proposed vaginal administration as a way to reduce the side-effects associated with danazol administration. When used postoperatively in deep infiltrative endometriosis, danazol 200 mg daily, administered vaginally for 12 months, showed a significant reduction in pain symptoms within 3 months, with efficacy throughout treatment and few side-effects [57]. The same route of administration reduced dyspareunia and vaginal bleeding in adenomyosis [58] (Fig. 1).

#### *3.1.4. Intrauterine device loaded with levonorgestrel*

Biopsy samples of endometriotic tissue from women treated with a levonorgestrel-releasing intrauterine system (LNG-IUS) for 6 months showed a decrease in the expression of glandular and stromal ER- $\alpha$ , ER- $\beta$  and PR, and a reduced cell proliferation index [59].

LNG-IUS reduced the recurrence of moderate or severe dysmenorrhoea for 1 year [60]. This finding was confirmed by prospective observational studies, and was seen in some cases with failed medical and surgical attempts to treat pain [61,62]. Longer follow-up periods of up to 3 years revealed a significant reduction in dysmenorrhoea scores, with up to 87.5% of patients who maintained the device reporting decreased pain [63]. Studies have compared the effectiveness of 6 months of therapy with LNG-IUS with GnRH agonists to treat endometriosis-related chronic pelvic pain, and a stronger pain-reduction effect was reported [64,65]. Long-term follow-up of these patients revealed that 59% were still using the device 3 years after its insertion, and the VAS score was  $\leq 3$  in 82.6% of these women [66]. Bleeding and pain were more frequently related to LNG-IUS

than GnRH agonists. However, with LNG-IUS, symptoms of the hypo-oestrogenic state were avoided and a better lipid profile was observed compared with GnRH analogues [67] (Fig. 1).

### 3.2. *Selective progesterone receptor modulators*

SPRMs interact with PRs to block or modify downstream effects. SPRMs have the potential for greater efficacy and flexibility than traditional treatments for endometriosis due to: (1) selective inhibition of endometrial proliferation without systemic effects of oestrogen deprivation; (2) reversible suppression of endometrial bleeding via a direct effect on endometrial blood vessels; and (3) the potential to suppress endometrial prostaglandin production in a tissue-specific manner.

In human cell lines, a number of SRPMs, including mifepristone, asoprisnil, ulipristal acetate, lonaprisan and telapristone acetate, suppress endometrial proliferation, resulting in endometrial atrophy. Further studies on animal models showed that mifepristone, onapristone and ZK136799 suppress endometrial growth and reduce the production of prostaglandins with possible benefits in terms of pain [68].

Modulators with potent progesterone antagonist activity, comprising the progesterone antagonist mifepristone and asoprisnil, have been proposed as therapeutic agents for endometriosis [69].

Only two small open clinical trials have been published using mifepristone for the treatment of endometriosis. Mifepristone 50 mg daily has been shown to improve pain and cause regression of endometriotic implants [70], but is unable to control the growth of endometriotic lesions at a lower dose [71]. Mifepristone-loaded subcutaneous implants may also be an effective treatment for endometriosis [72].

A randomized placebo-controlled clinical trial using asoprisnil for the treatment of endometriosis-associated pain significantly reduced the average daily combined non-menstrual pelvic pain/dysmenorrhoea scores compared with placebo, and had a favourable safety and tolerability profile over the 3-month treatment period [73]. The trials on this drug were stopped for

some cases of endometrial hyperplasia. Indeed, SPRMs induce endometrial changes known as ‘PR-modulator-associated endometrial changes’. The levels of oestrogens are maintained, and bone mineral density is not affected. Common side-effects of SPRMs are headache, abdominal pain and tenderness.

SPRMs may offer a pharmacological therapeutic alternative to improve the symptoms of endometriosis by the suppression of ovulation, antiproliferative effects on the endometrium and suppression of endometrial bleeding [74] (Fig. 1).

#### **4. Ovulation inhibition**

Oestroprogestins (EPs) inhibit gonadal oestrogen production through negative hypophyseal feedback inducing ovarian suppression. They may also reduce prostaglandin production secondary to endogenous oestrogens, decreasing the inflammatory status. Due to better tolerability and lower metabolic impact, cyclic and continuous EPs are considered as effective chronic treatment for endometriosis, either as a strategy to avoid surgery or as a postoperative adjuvant therapy to prolong the symptom-free interval [8]. EPs improve both dysmenorrhoea and non-menstrual pelvic pain. Long-term regimens of either continuous or cyclic EPs reduce the frequency and severity of recurrent dysmenorrhoea [75]. Continuous EP therapy is better than cyclic EP therapy for improving pain. There is no consensus on the role of EPs for the prevention of endometriosis.

#### **5. Conclusion**

Hormonal treatment of endometriosis is based on the concept that the ectopic endometrium is modulated by sex steroid hormones and undergoes the same cyclic changes as eutopic endometrium. The main aims are to reduce oestrogen levels (systemically and locally) and to restore PR resistance. These strategies aim to reduce the tropism of endometrial lesions or to generate a pseudo-decidualization, avoiding the cyclical changes of the endometrium and therefore subsequent menstruation and inflammation at the ectopic site.

An ideal hormonal therapy for endometriosis should be able to ameliorate pain, avoid a hypo-oestrogenic state and restore fertility, with limited side-effects, lower cost, and suitable for long-term administration. A possible future strategy could include the use of non-hormonal drugs associated with hormonal treatments to target multiple sites of action.

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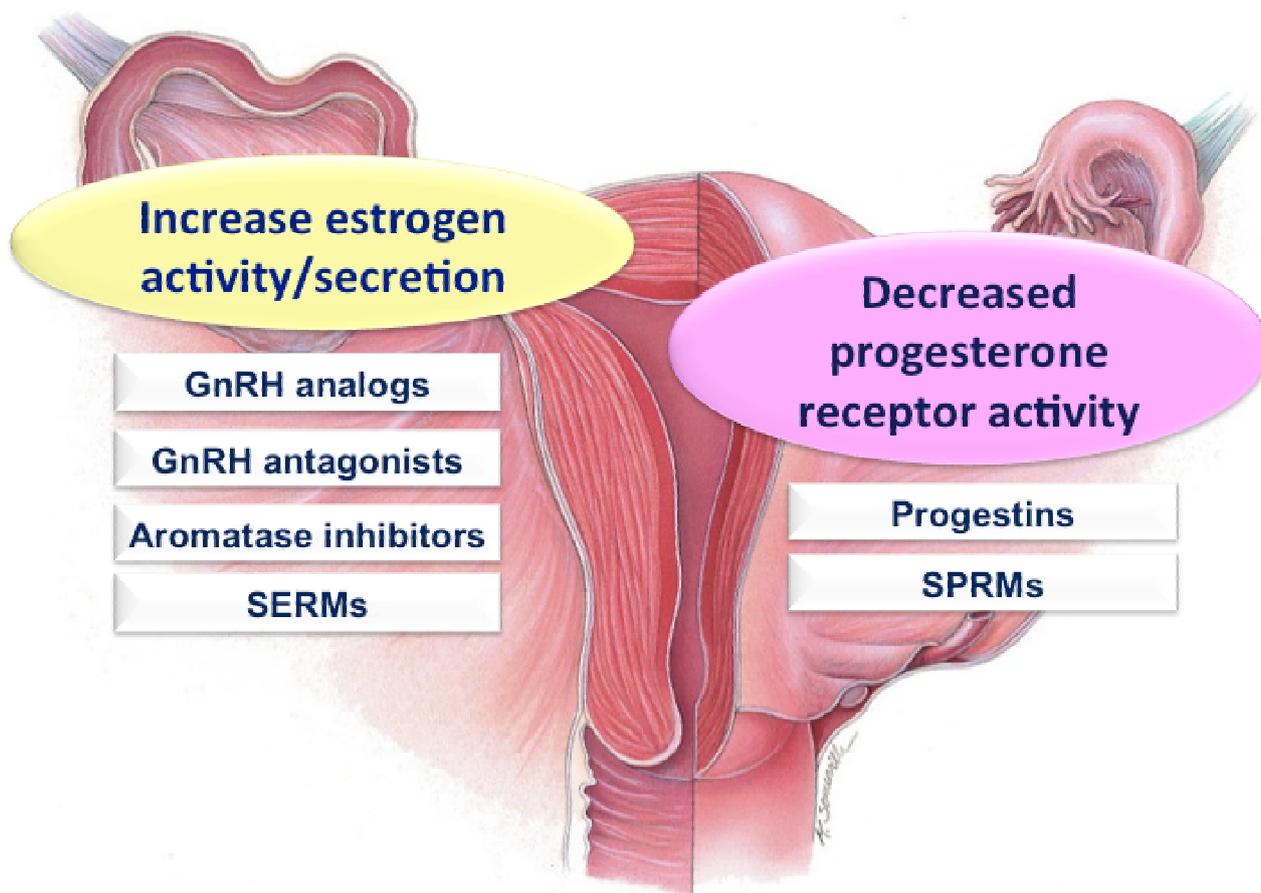
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**Fig. 1.** Oestrogen activity/secretion and progesterone receptor activity are the main targets for hormonal treatment of endometriosis.



#### Author queries

This article has been edited extensively - please check the proofs carefully to ensure that your meaning has not been altered

Section 3.1.2, para 2 – ‘because the percentage of irregular bleeding is statistically lower, confirmed by another study, its overall efficacy, tolerability and safety’ – please clarify

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Section 3.1.3, para 3 – ‘Danazol-loaded intrauterine systems [75] and danazol-loaded vaginal rings [75] significantly decrease dysmenorrhoea, dyspareunia and chronic pelvic pain in women with deeply infiltrating and rectovaginal endometriosis nodules are reduced [75].’ Please clarify

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