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Unopposed estrogens: current and future perspectives

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Abstract. – Estrogens and progestogens act on female reproductive tissues in opposite ways. As they counteract each other actions, the correct balance between these two classes of hormones is pivotal to avoid dangerous states. Unopposed estrogens occur when progestogen levels do not balance estrogens, primarily deriving from overproduction of estrogens via aromatase enzyme. In the endometrium, unopposed estrogens induce proliferative or invasive phenomena, which represent the first step toward different diseases. These pathologies include endometrial hyperplasia, endometrial polyps, endometriosis and adenomyosis. Endometrial hyperplasia and polyps are proliferative pathologies, while endometriosis and adenomyosis are characterized by the invasion of other tissues by endometrial cells. Current pharmacological treatments include Gonadotropin-Releasing-Hormone analogs, aromatase inhibitors and progestogens, either alone or in combination with estrogens. As these drugs usually lead to burdensome undesired effects, researchers seek to find new therapeutical molecules. Recent literature highlights the positive effects of metformin, an insulin sensitizing drug that reduces the insulin proliferative stimulus on the endometrium. D-chiro-inositol is an insulin second messenger with insulin sensitizing and mimetic properties, recently described as an aromatase down-regulator. Based on current evidence, D-chiro-inositol may be useful to treat the pathologies responsive to unopposed estrogens.

Key Words:
Estrogen, Endometrial hyperplasia, Endometrial polyps, Endometriosis, Adenomyosis, Inositol, D-chiro-inositol.

Introduction

Estrogens and progestogens are two classes of steroidal hormones involved in the regulation of several physiological processes[1,2], including the menstrual cycle, thus playing pivotal roles in female fertility[3,4]. If on the one hand estrogens are necessary throughout the entire menstrual cycle, on the other hand progestogens are mainly produced in the luteal phase, i.e.: the second half of the menstrual cycle.

Progestogens are directly synthesized from cholesterol and then converted in corticosteroids or in androgens (Figure 1)[5]. Corticosteroid pathway the proceeds toward mineralocorticoids and glucocorticoids. In the androgen pathway, instead, further enzymatic reactions lead to the production of androstenedione and/or testosterone. They are then converted in estrogens, in reactions mediated by an enzyme of the cytochrome family called aromatase (Figure 1)[6].

Estrogen and Progestogen Unbalance

Steroidal hormones are strongly related to each other in terms of functions and chemical structures (Figure 1)[5]. However, estrogens and progestogens mediate different, sometimes opposed activities. In the uterus, in fact, estrogens promote cellular growth and proliferation, while progestogens induce cellular differentiation[7]. Thus, the balance between estrogens and progestogens is paramount to avoid potentially dangerous hormonal stimulations. Either high levels of estrogens associated with normal levels of progestogens, or low levels of progestogens coupled with normal levels of estrogens[6] result in a relative estrogen overstimulation. The condition is known as *unopposed estrogens* and represents a major risk factor for several pathologies deriving primarily from proliferation and invasiveness[8-11]. While the effects

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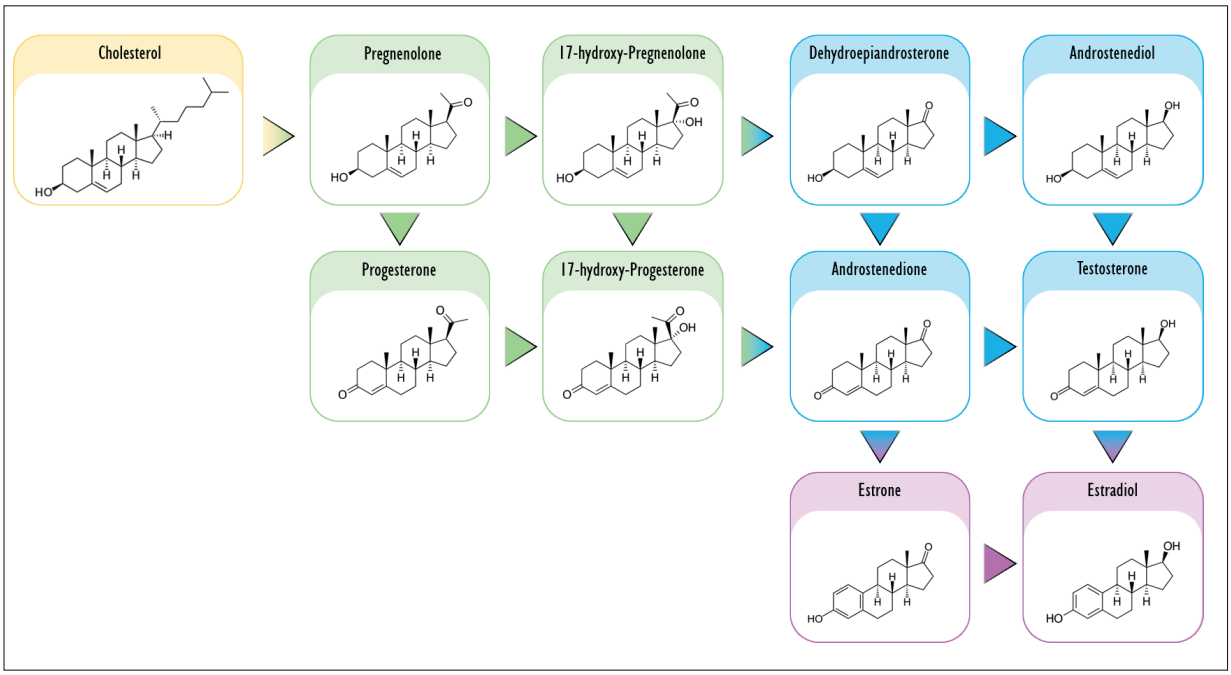

Figure 1. The steroidogenesis pathways.

of unopposed estrogens in cancer pathogenesis and progression, including uterine and breast tumors are widely described[6,12], the literature lacks recent publications that gather comprehensive evidence on their role in other gynecological conditions. On these premises, the present review aims to provide an updated report that focuses on the following aspects: 1) uterine benign formations associated with unopposed estrogens, including endometrial hyperplasia and polyps; 2) delocalization of non-cancerous uterine tissues, namely endometriosis and adenomyosis, that depends on unopposed estrogens.

Estrogen and Progestogen Receptors

In women estrogens are primarily produced by the ovaries, while progesterone is produced by the adrenal cortex and the corpus luteum. Once secreted, steroidal hormones exert their final effects in target cells that express the related receptors[3,4]. Estrogen signaling is transduced either *via* G-protein-coupled estrogen receptor (GPER) on the membrane or *via* water-soluble receptors (ER), named ERα and ERβ[2,4,13,14]. Likewise, progesterone receptors make up a heterogenous family. Canonical progesterone receptor is detectable in two isoforms named Progesterone Receptor A (PR-A) and B (PR-B), the latter providing the stronger downstream response[1,3]. Other progesterone receptors include those located in cell membranes, which are divided in two families, namely membrane progesterone receptors (mPR)[15] and progesterone receptor membrane components (PGRMC)[16]. Overall, locally altered expression of estrogen and/or progesterone receptors may account for the effect of unopposed estrogens in pathological conditions[17-23].

Endogenous and Exogenous Hyper-estrogenic Stimulation

Other than from altered receptor expressions, hyperestrogenism can derive either from diminished progesterone or from an augment of estrogens. The latter, known as hyperestrogenism or absolute hyperestrogenism, generally derived from the altered expression of the aromatase enzyme. Aromatase, encoded by the gene Cyp19a1, is expressed in different tissues, including ovaries, adrenal glands, placenta, some neuronal species, and white adipose tissue (WAT). Considering this, obesity represents an important risk factor, as it may play a major role in estrogen production. Moreover, as the mammalian body accumulates WAT, the more severe the obesity, the higher the production of estrogens[24].

Even if the precise etiology of unopposed estrogens remains unexplained in most cases, some conditions may constitute a risk factor. Ageing is one of the most important, as it plays a crucial role in endogenous overproduction of estrogens. O' Connor et al[25] reported that during the reproductive age, women are physiologically exposed to different grades of unopposed estrogen. In detail, women of childbearing age are exposed to unopposed estrogens for at least forty days every six months, with the number of days that increases toward menopause. During the perimenopausal transition, women are exposed to unopposed estrogens for almost twice the number of days. Once in menopause, unopposed estrogen stimulus ends and, as a consequence, the risk of developing associated pathologies decreases. On the other hand, reduced estrogens during menopause may cause other issues, as bone loss or vaginal dryness, and Hormone-Replacement Therapies (HRT) are often prescribed[26-28]. Unfortunately, HRT expose women to unopposed estrogen stimulus even after menopause, reiterating the issue.

Other drugs can mimic unopposed estrogen stimulus. Among them, the class of Selective Estrogen Receptor Modulators (SERMs) is one of the most studied[2,29-31]. As their name suggests, SERMs exhibit different activities depending on the specific receptor and the involved tissue. For example, Tamoxifen is a widely prescribed SERM, currently used to treat breast cancer[29]. In the breast, tamoxifen binds ER and induces the expression of progesterone receptor, while reducing the growth of the tumoral mass. Thus, tamoxifen acts both as antagonist and agonist in the same tissue, probably depending on the specific receptor[2]. However, in the uterus, tamoxifen treatment mimics a strong unopposed estrogen stimulus as the agonist activity on ER seems to prevail. The effect is an excessive stimulus that leads to deregulated proliferation (Figure 2)[29-31].

Selective Progesterone Receptor Modulators (SPRMs) are another class of molecules mimicking unopposed estrogen stimulus. SPRMs act at the same time both as agonist and antagonist of the PR, partially mimicking the effect of progestogens and inhibiting other downstream effects. Even if such phenomenon is still unclear, SPRMs may exert both activities in the uterus, and in the case of antagonist dominance they mimic unopposed estrogen effects[32]. Among SPRMs, Ulipristal Acetate (UPA) is widely used in the treatment of uterine fibroids or to induce pharmacological abortion. UPA may induce cellular proliferation that histologically resembles the morphological effects induced by unopposed estrogens, thickening the endometrium (Figure 2)[33-35].

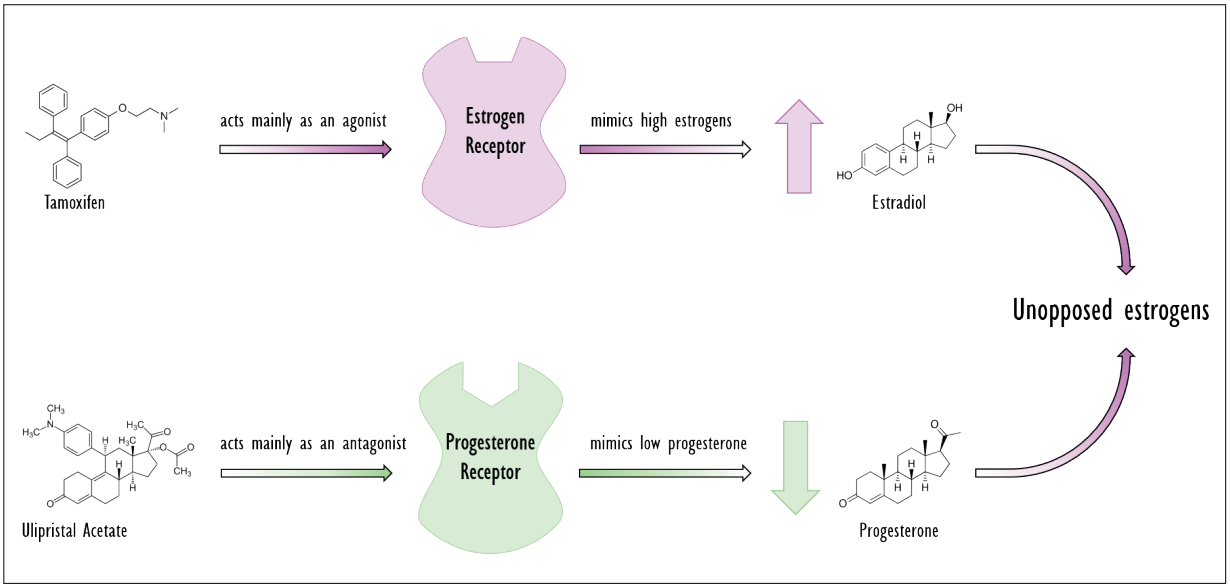

Figure 2. The effects of Selective Receptor Modulators on estrogen and progesterone receptors in the uterus that can mimic unopposed estrogens.

Table I. Main uterine benign pathologies that respond to unopposed estrogens and the relate symptoms.

Effect of estrogen stimulus	Pathologies	Main symptoms
Proliferation	Endometrial hyperplasia	Abnormal uterine bleeding
		Heavy menstrual bleeding
		Pelvic pain
		Menstrual cycle <21days
	Endometrial polyps	Pelvic pain
		Dysmenorrhea
		Nausea
		Pain during sexual intercourse
		Reduced fertility
Invasiveness	Endometriosis	Abnormal uterine bleeding
		Heavy menstrual bleeding
		Reduced fertility
	Adenomyosis	Pelvic pain
		Dysmenorrhea
		Abnormal uterine bleeding
		Reduced fertility

Pathologies

Unopposed estrogens may cause different pathologies (Table I), depending on several factors. Indeed, the systemic levels of estrogen and progesterone may impact the endometrium in different ways, promoting the proliferation and the invasiveness of the tissue[6]. Moreover, the levels of estrogen and progesterone receptors may also contribute to mimic a condition of unopposed estrogen, although local and systemic levels of hormones are in the normal range[21-23]. Thus, depending on the hormonal stimuli and the expression of the receptors, unopposed estrogens may cause pathologies characterized by either hyperproliferation or invasiveness (delocalization).

Hyperproliferation

Estrogens play important roles in cellular proliferation of various tissues, acting as growth factors. Several body districts are responsive to the estrogen stimulus, including reproductive and bone tissues. Notably, the unopposed estrogen condition can induce a higher proliferation of uterine tissues, especially of the endometrium, which highly expresses estrogen receptors[6]. This estrogen-induced growth appears at the beginning as an endometrial thickening, which can further evolve into proliferative pathologies[36]. Endometrial hyperplasia and endometrial polyps are the most investigated proliferative pathologies of the endometrium.

Endometrial Hyperplasia

Endometrial hyperplasia (EH) is a pre-cancerous, non-invasive proliferation of the endometrial tissue characterized by increased volume and relative abundance of the glandular component compared to the stroma[36]. As it derives from an endometrial thickening, ultrasound diagnosis is based on the detection of thickened endometrium, usually during routine visits; following biopsy allows to confirm the hyperplasia and investigate the subtype[37]. In fact, in 2014, WHO recognized two different types of EH, namely 1) hyperplasia without atypia and 2) atypical hyperplasia, which features cellular atypia or supernumerary nuclei[38]. Hyperplasia without atypia does not include significant genetic alterations, so the rate of malignant transformation is relatively low (1-3%). On the contrary, atypical hyperplasia displays higher risk of malignant transformation, estimated to be in the range 25-59%[38].

EH mostly affects women between the age of 45 and 69, with a peak of diagnoses between 50 and 54, which accounts for almost 400 diagnoses per 100,000 women every year. Overall, in women aged 18-90, the estimated incidence is greater than 133 diagnoses per 100,000 women every year[39]. The estimated prevalence in the population displaying physiological bleeding patterns is lower than the prevalence on aggregate, reaching only 6%. In detail, the greater part (5% of total population) has EH without atypia, while the remainder displays EH with atypia[39].

The etiology of both types of EH is strongly dependent on proliferative stimuli. Estrogens

represent the strongest proliferative stimulus in the endometrium, accounting for most of the EH cases. Moreover, as endometrial hyperplastic tissue shows reduced expression of PR-A and PR-B, it fails to respond properly to progestogens, exacerbating the estrogen effect[21,36,40].

Obesity is an important risk factor for EH due to overproduction of estrogens and development of insulin resistance, leading to compensatory hyperinsulinemia[36]. Insulin, indeed, strongly stimulates cellular proliferation by binding to the receptors of both insulin and insulin-like growth factor (IGF)[41], thus contributing to EH. Moreover, receptors for IGF and Estrogen show cross-talk phenomena, enhancing each other responsiveness[42].

Abnormal uterine bleeding is the most common symptom of EH, and encompasses intermenstrual bleeding, irregular bleeding, and postmenopausal bleeding. The latter is one of the most important in the diagnosis, as approximately 15% of women displaying postmenopausal bleeding suffer from EH, generally induced by pharmacological treatments such as HRT or tamoxifen. Other relevant symptoms include heavy menstrual bleeding, generally coupled with moderate to severe pain, and short menstrual cycles defined by less than 21 days between periods[43] (Table I).

Endometrial Polyps

Endometrial polyps are localized proliferative masses displaying fibrous areas that protrude in the uterine cavity. They consist of both a glandular and a stromal component, with high vascularization of the area[9]. As for EH, the gold standard for the diagnosis of polyps is the ultrasonography. Hysteroscopy with biopsy allows for further histological investigations[44]. Polyps also share some molecular signatures with EH, exhibiting higher expression of ER compared to healthy endometrial tissue. In this case PR expression appears increased as well, in contrast to what happens in EH[18,45].

Polyps are generally benign formations, with a malignant transformation rate lower than 3%[46]. They are strongly susceptible to the unopposed estrogen stimulus; indeed, they usually occur as the most frequent side effects of tamoxifen treatment[47,48]. Epidemiological analyses estimate a global prevalence ranging from 7% to 12% in the general population, rising to 27% in women on tamoxifen[46,49,50]. Also, UPA and HRT may cause endometrial polyps[35,47]. Drugs account for the greater part of diagnosed polyps of known origin[47]. However, the majority of diagnosed polyps remain of uncertain cause since a univocal mechanism for the onset still remains unidentified. Other risk factors include ageing and obesity, as for EH[49,51]. Of note, other than acting as ER agonist in the uterus, tamoxifen can also improve IGF signaling, providing an increased growth stimulus[52].

As for EH, abnormal uterine bleeding is frequently associated with polyps, occurring in up to 68% of patients[44]. It includes irregular menses, menorrhagia, intermenstrual bleeding, and postcoital bleeding. Thirteen to 50% of the abnormal bleeding events are attributable to polyps[44]. A recent review[44] concluded that 44% of postmenopausal women with polyps display bleeding, rising to 82% in affected premenopausal women (Table I).

Reduced fertility often occurs in premenopausal women with polyps. Even if the causes are unclear, the most likely explanations include obstruction of the uterus or biochemical effects that reduce the chance of a correct embryo implantation[44].

Delocalization

Estrogens and progestogens support two opposite processes in the endometrium. While the former induces epithelial-mesenchymal transition (EMT), the latter sustains mesenchymal-epithelial transition (MET)[53-56]. These events are deeply investigated in the study of cancer metastasis, but they seem involved also in other gynecological pathologies, including endometriosis and adenomyosis.

Endometriosis

Endometriosis consists in the ectopic presence of endometrial tissue outside the uterus, generally in the adjacent intraperitoneal cavity, even if "distal" endometriosis occurs in some cases[10,57]. Ectopic islets of endometrial tissue respond to the hormonal signal of estrogens and progestogens[10,58] and undergo all the physiological changes occurring in the eutopic endometrium during the menstrual cycle, including cyclic bleeding[10,57]. Endometriosis may interest ovarian tissues, exposing patients to a higher risk of developing ovarian cancer[10].

Although the involvement of absolute levels of estrogens in the etiopathogenesis of endometriosis is still debated, the role of unopposed estrogens is well documented in such process, causing cell proliferation and pain[22]. Indeed, literature reports that endometriotic tissues express aromatase, locally producing estrogens to sustain self-growth[59,60]. Furthermore, data collected in the last decade indicate a possible involvement of insulin and IGF in the pathogenesis of endometriosis[61-63].

Recent evidence[64] indicates that the incidence of the pathology in fertile-age women is constantly around 72 per 100,000 women every year. Estimations on the prevalence indicate that endometriosis affects around 10% of women aged 15 to 54, even if a clear diagnosis is difficult to achieve[10] as the only unbiased diagnostic method is the surgical visualization. Projections estimate that more than 190 million patients currently suffer from endometriosis worldwide[65]. Endometriosis affects up to 50% of infertile women and 21% of women reporting pelvic pain[66,67]. Conversely, pelvic pain unrelated to menses affects more than 65% of women with endometriosis, while menstrual pain affects up to 93% of them[68], leading to an over-prescription of painkillers[69].

Other common symptoms are gastrointestinal disorders as nausea or vomiting, reported respectively by more than 50% and 15% of the women[68]. According to the National Institute of Health (NIH), other gynecological and gastrointestinal symptoms and signs include pain during sexual intercourse, intestinal pain, painful bowel movements, painful urination, and infertility[70] (Table I).

Adenomyosis

Adenomyosis consists in the ectopic occurrence of endometrial tissue specifically in the myometrium, and thus inside the uterus. As expectable, endometriosis and adenomyosis are associated: about 50% of women suffering from endometriosis exhibit adenomyosis as well[71]. In some cases, adenomyotic tissue can aggregate in a disorganized mass named adenomyoma. This tumoral mass consists of proliferating endometrial and myometrial cells that compress surrounding tissues[72]. To date the diagnosis is still based on histological analyses following hysterectomy, even though recent progresses in imaging techniques allow an easier diagnosis[72]. Histological analyses revealed that several proteins involved in estrogen metabolism appear altered in adenomyosis. Specifically, cellular expression levels of ER are increased, while decreased response to progesterone fails to counterbalance the response to estrogens[23,73,74]. As in endometriosis, adenomyotic tissue displays high expression of aromatase, producing its own growth stimuli[60].

Adenomyosis has a prevalence of 0.79% on aggregate, ranging from 0.02% in women younger than 20 years to 1.54% in women aged 41-45[75]. A study[76] revealed that adenomyosis is probably underestimated, as routine exams still lack histologic examinations for the diagnosis. A large study in the US estimated that the incidence of the disease is around 289 diagnoses every 100,000 women every year. The same study pointed out that women aged between 41 and 45 years are the most affected, with incidence above 600 every 100,000 women every year. This may be ascribable to a longer physiological exposure to estrogens, which is a known risk factor for the pathology[75]. Other important risk factors include high body mass index (BMI), probably due to the synthesis of estrogens by the WAT, and the occurrence of an early menarche that extends the duration of estrogen exposure[11]. Recent evidence[77] on altered intracellular signal of IGF highlighted that impaired insulin signal may also account for the relationship between elevated BMI and adenomyosis[77]. Previous data suggested that tamoxifen treatments may represent an important risk factor for the development of adenomyosis[78].

Pelvic intermenstrual pain and dysmenorrhea are the most prevalent symptoms of adenomyosis, affecting up to 93% of the patients[79], and women with adenomyosis also seem to have a higher incidence of abnormal uterine bleeding. In fact, up to 49% of women displaying abnormal uterine bleeding exhibit adenomyosis[79]. The condition may reduce fertility because of alterations in both endometrium and inner part of myometrium (Table I).

Current and Future Perspectives

Current treatments for all the pathologies mentioned above include surgical and pharmacological intervention. Even if surgery is often the only definitive treatment, it may reduce or altogether preclude fertility, and is generally avoided when possible. Accordingly, the latest research focused on pharmacological treatments that reduce the estrogen relative stimulus to manage the symptoms.

Surgical Treatments

Endometrial Hyperplasia

Surgical approaches for hyperplasia without atypia include ablation, laser therapy or endometrial resection. Although ablation and laser therapy are considered safe and effective, hysterectomy is the treatment of first choice unless the patient wishes to preserve fertility. Conversely, in the case of atypia, hysterectomy is highly recommended to avoid malignant degenerations[36,80].

Endometrial Polyps

Hysteroscopic resection of the polyp, also known as polypectomy, is a non-invasive outpatient procedure considered as gold standard, unless the polyps are malignant[81]. In this latter case, hysterectomy is highly recommended to avoid complications and recurrencies[81]. In fertility-sparing procedures, the recurrence risk of endometrial polyps reaches 20% in the first two years following surgery, and post-operative pharmacological management may be beneficial[82].

Endometriosis

Surgical approach in endometriosis is nowadays controversial. Surgery can effectively remove the endometriosis foci, but removal may be incomplete due to the dispersion of the lesions. Moreover, the outcome often depends on the experience of the surgeon[83].

Adenomyosis

Hysterectomy is the treatment of election but fertility-sparing procedures (e.g., adenomyomectomy and high intensity focused ultrasound) are the first choice in case of patients in the reproductive age[84].

Pharmacological Treatments

Endometrial Hyperplasia

In the case of EH without atypia, pharmacological approaches are nowadays the best choice to reduce the risk of progression and recurrence, while preserving fertility[80]. Earlier options included agonists of the Gonadotropin-releasing Hormone (GnRH), which induce menopause as side effect[36]. Third generation aromatase inhibitors (AIs) represent an interesting therapeutic opportunity that preserves fertility[85,86], but they usually have significant unwanted effects, including the loss of bone density[87]. For this reason, scientists searched for novel treatments devoid of side effects. Nowadays, progestin is the treatment of election to reduce cell proliferation and concomitantly avoid the surgery in the case of EH without atypia, without affecting women's fertility[88-90]. Despite their efficiency, progestins also can expose to undesired effects, including gastrointestinal disturbs, dizziness, weakness, pain, difficult breathing and chest pain[91]. Among the emerging treatments for EH, metformin is an insulin sensitizer that represents a new option currently under investigation. Research on insulin sensitizing compounds, indeed, highlighted their effectiveness in such clinical picture, even if the mechanism of action is unrelated to estrogens[92]. A recent meta-analysis investigated the efficiency of metformin alone or associated with progestins *vs.* the canonical progestin treatment[93]. The results suggest that metformin could represent both an efficient treatment and an interesting add-on therapy to progestins to counteract stimuli of proliferation. However, also metformin may cause serious undesired effects including disturbs of the digestive system, anemia, deficiency of vitamin B12 and hypoglycemic episodes[94,95].

Polyps

Progestins are used to treat the polyps without surgical intervention and to reduce the risk of recurrence after polypectomy[82,96]. Most importantly, progestins help preventing the onset of endometrial polyps in patients taking SERMs for breast cancer[90,97,98]. AIs represent a relevant pharmacological strategy as they reduce the endometrial thickness in oncological patients previously on tamoxifen[99].

A recent clinical trial[100] demonstrated that metformin exhibits inhibitory activity on the occurrence of tamoxifen-induced endometrial polyps, reducing both estrogen synthesis and insulin stimulus[100].

Endometriosis

While surgical intervention is an option, pharmacological approaches that reduce the symptoms are generally preferred. Moreover, they can also reduce the risk of post-surgical recurrence[101]. As pathogenetic mechanisms that cause endometriosis are still unclear, these treatments reduce the estrogen stimulus. They include progestins, combined oral contraceptives, GnRH agonists, AIs and metformin. Progestins balance estrogen overstimulation,

significantly improving symptomatology[102-108]. Combined oral contraceptives are effective in reducing symptoms and improving the quality of life, especially regarding the non-cyclical pain[109]. GnRH agonists reduce estrogen synthesis but induce menopause[110]. AIs block the synthesis of estrogens and effectively reduce both the dimension of the lesions and the symptoms, even if evidence on their use is still limited[111-113]. Metformin use is supported by pre-clinical and *in vitro* robust evidence on its molecular efficacy[114-119]. A single clinical trial exists[120] and reports great improvements in pain recovery, dysmenorrhea, and infertility after three months, improving further after six months of metformin therapy. Among all the therapeutic opportunity, progestogens and combined oral contraceptives remain the treatments of choice to date, despite their side effects[91,121].

Adenomyosis

The use of GnRH analogs is an effective approach to reduce symptoms, but fertility is compromised in this case as well[122]. Lately, treatments with progestins and AIs have been investigated to reduce respectively pelvic pain and dysmenorrhea, without compromising fertility. They proved effective in relieving symptoms, and in reducing uterine volume[123]. Recent *in vitro* studies indicate that metformin can be used to treat adenomyosis[124], and clinical evidence[125] indicated that it improves the outcomes of ultrasound treatments.

Excluding GnRH analogs, these pharmacological treatments for pathologies associated with unopposed estrogens may help preserve the fertility. However, they are associated with adverse effects. Indeed, progestins may provoke disturbs of the gastrointestinal tract, weakness or episodes of hyperglycemia[91]. In addition to disturbs of the digestive system, AIs may induce the loss of bone density, eventually leading to arthrosis or osteoporosis. They can also cause breast and abdominal pain, the latter usually coupled with abnormal bleeding[87]. Metformin is gaining attention in the research on the pathologies here described. However, its use may be accompanied by disturbs of the gastrointestinal tract, muscle pain, chest pain, stomach pain, and heartburn[94]. For these reasons, novel treatments that modulate the estrogen stimulus without causing relevant side effects are highly desirable and constitute a current field of research.

Future Perspectives: D-Chiro-Inositol

Recent findings highlighted the clinical interest for D-chiro-inositol, a molecule acting both as insulin sensitizer and as transcriptional inhibitor of aromatase[5,126-129]. D-chiro-inositol is a natural polyol belonging to the inositol family. Detectable in almost every form of life, inositols are hydrophilic components of phospholipid bilayer in animals, and participate in different regulatory pathways, including insulin signaling[130]. Among nine possible stereoisomer, myo-inositol is the most abundant in nature, while D-chiro comes second. They account for different, although sometimes related, activities[129] investigated both *in vitro* and *in vivo*[131]. Inositols are also safe compounds, with null or minor side effects, and the FDA included them in the list of Generally Recognized as Safe (GRAS) molecules[132].

In recent years, D-chiro-inositol gained interest for its two-sided activity, both on metabolic and steroidogenic pathways. Metabolic activities of D-chiro-inositol are known since its early characterization. Phosphoglycans containing D-chiro-inositol were first identified as physiological second messengers of insulin signaling pathway by Larner et al[133]. Further evidence from the same research group highlighted that insulin stimulus in fibroblasts prompted the biosynthesis of D-chiro-inositol from myo-inositol. Such conversion occurs at different grades in all insulin-responsive tissues *via* an insulin-stimulated epimerase enzyme[134,135]. They also found out that diabetic patients displayed reduced D-chiro-inositol levels in the urine, suggesting an impairment of intracellular synthesis[136]. Subsequent pre-clinical and clinical research confirmed the insulin-sensitizing properties of such molecule[137-141]. Clinical evidence also revealed that insulin sensitizers as metformin act mainly by prompting the release of D-chiro-inositol from membranes[142].

The importance of inositols as second messengers of sexual hormones is a fairly recent finding. Nestler et al[143] first demonstrated the role of D-chiro-inositol in the steroidogenesis, pointing out that insulin inhibits estrogen synthesis *via* inositol-glycans. Later, Sacchi et al[126] analyzed the effects of *in-vitro* D-chiro-inositol treatment on human granulosa cells from healthy subjects, demonstrating that it inhibits the expression of aromatase in a dose-dependent manner. Interestingly, such activities define a unique and specific role for D-chiro-inositol in clinical practice, representing a therapeutic opportunity in case of

excess estrogens. Recent literature highlights that D-chiro inositol displays different effects depending on the patient, and the dosage and timing of treatments[129,131,144]. Particularly, low doses seem to favor its metabolic effects, while higher dosages allow the effect on steroidogenesis[129,131]. As treatments with D-chiro-inositol traditionally involved insulin-resistant patients, in which the metabolic effect is predominant, this feature only emerged in recent years.

Clinical trials with male subjects treated with D-chiro-inositol highlighted that high doses promote androgen accumulation, while repressing estrogen synthesis in non-insulin-resistant patients. Indeed, Monastra et al[145] pointed out that healthy volunteers exhibit reduced levels of estradiol and estrone and increased levels of testosterone after 30 days of treatment with 1000 mg per day of D-chiro-inositol. Moreover, Nordio et al[146] treated elderly hypogonadal males with 1200 mg per day of D-chiro-inositol for 30 days, obtaining significant results both in reducing estrogens and in increasing androgens. Despite such encouraging evidence in males, data on clinical effectiveness of D-chiro-inositol in women are still unavailable.

The recovery of physiological levels of estrogens and progestogens would result in important effects: halt the progression of the pathologies and reduction of the associated symptoms, improving the quality of life of women with conditions worsened by unopposed estrogen stimulus. Data indicate that D-chiro-inositol reduces both insulin stimulus and estrogen production, mimicking the effects of a treatment with metformin and AIs but without the same adverse effects. Due to its activity on the expression of aromatase and on insulin signaling, D-chiro-inositol could be helpful to reduce systemic estrogen production, and to lower insulin levels, thus removing pivotal growth signals[129,147]. As some of these pathologies display *in situ* increased expression of aromatase, D-chiro-inositol could represent an efficient opportunity by reducing local production, thus restoring a balance. Some detrimental effects for D-chiro-inositol are reported only in specific cases including treatment with high dosages for long periods of time[142].

Conclusions

Unopposed estrogens, which may arise from multiple conditions, negatively affect the life of women, and expose them to the risk of estrogen-responsive pathologies of the endometrium. In particular, endometriosis, adenomyosis, endometrial hyperplasia, and polyps are estrogen-responsive pathologies that may heavily impact the quality of women's life. Current treatments include surgery, which is usually invasive and precludes fertility, and drugs that may reduce fertility as well and expose patients to undesired effects. Accordingly, new therapeutical options should be evaluated. Recent literature reports the potential benefits of D-chiro-inositol supplementation. Based on data available in the literature, D-chiro-inositol treatment could reduce circulating estrogens and increase insulin sensitivity, thus representing an ideal approach for women suffering from estrogen-responsive conditions, especially in the case of excess estrogen stimulus. Considering the properties and clinical effects of D-chiro-inositol, proper clinical trials are warranted to establish its efficacy in conditions connected with unopposed estrogens.

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MMO, CA and VU conceptualized the work. MMO, RG, GF, GP and VU searched literature for appropriate articles. RG, GF and GP drafted the original paper. MMO, GF, CA and VU critically revised the article. All authors read and approved the final version of the paper.

Conflict of Interest

RG, GF and VU are employed at Lo.Li Pharma. MMO, GP and CA declare no conflict of interest.

Data Availability

Not applicable.

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