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# RESHAPING PURPOSE - USE OF METFORMIN IN ENDOMETRIOSIS AN APPROACH TO RECENT LITERATURE

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**ABSTRACT:**
*ENDOMETRIOSIS CAN BE CLASSICALLY DEFINED AS THE PRESENCE OF A FUNCTIONAL ENDOMETRIAL LAYER WITH ENDOMETRIAL GLANDS OUTSIDE THE UTERINE CAVITY, INDUCING A CHRONIC, INFLAMMATORY REACTION, LINKED TO CHRONIC PELVIC PAIN AS WELL AS FERTILITY ISSUES. A LARGE AMOUNT OF LITERATURE SURVEYS AS WELL AS CURRENT STUDIES RECKON ENDOMETRIOSIS AS THE RESULT OF A DEFECTIVE IMMUNOSURVEILLANCE*

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*CASCADE IN FERTILE WOMEN, SUGGESTING THAT THE CONSTANT PERITONEAL INFLAMMATORY ENVIRONMENT STIMULATES PROGRESS AS WELL AS MAINTENANCE OF THE DISEASE. THEREFORE, NOVEL THERAPEUTICAL APPROACHES ARE NEEDED IN ORDER TO TARGET DIFFERENT PATHOGENIC SPECTRUMS. METFORMIN, A WIDELY USED BIGUANIDE IMPROVING INSULIN SENSITIVITY IN DIABETES, CAN ALSO PROVIDE BOTH ANTI-INFLAMMATORY PROPERTIES AS WELL AS A MODULATORY EFFECT ON OVARIAN STEROID PRODUCTION, TWO ACTIONS THAT HAVE BEEN SUGGESTED TO OFFER NEW PERSPECTIVES IN ENDOMETRIOSIS THERAPY. MANAGING ENDOMETRIOSIS REQUIRES TARGETING A COMPLEX NETWORK OF LOCALLY PRODUCED CYTOKINES MODULATING THE GROWTH AND INFLAMMATORY BEHAVIOR OF ECTOPIC ENDOMETRIAL IMPLANTS. THEREFORE, WHAT WE DEFINE AS THE MAIN OBJECTIVES OF THIS LITERATURE REVIEW BECOME AS FOLLOWING: TO ASSESS WHETHER METFORMIN MAY BE EFFECTIVE AS A NOVEL APPROACH FOR ENDOMETRIOSIS, DOSE-DEPENDENTLY OR NOT, AS WELL AS TO EVALUATE THE EFFECTS OF THIS AGENT ON INFLAMMATORY RESPONSES, ESTRADIOL PRODUCTION AND NEVERTHELESS ENDOMETRIOTIC STROMAL CELLS PROLIFERATION IN BOTH IN VITRO AND IN VIVO CONDITIONS.*

**KEY WORDS:** ENDOMETRIOSIS, METFORMIN, IL-8, IL-6, IMMUNOSURVEILLANCE, VEGF

**BACKGROUND:**

It is a well acknowledged fact that endometriosis can be defined as the presence of functional endometrial glands and stroma within the pelvic peritoneum and other extrauterine sites, inducing a chronic inflammatory reaction linked to pelvic pain and infertility[10]. It is estimated that it affects 2–10% of women in the reproductive age group. Endometriosis is viewed to be a polygenically inherited disease of complex multifactorial etiology showing a wide range of immunological surveillance issues as well as a wide spectrum of cytokines to be involved[11]. Sampson's theory regarding the transplantation of endometrial tissue on the pelvic peritoneum via retrograde menstruation is the most widely accepted explanation for the development of pelvic endometriosis[12]. Since retrograde menstruation is observed in almost all reproductive-aged women undergoing menstrual bleeding, endometriosis is postulated to develop as a result of the co-existence of a defect in clearance of the menstrual efflux from pelvic peritoneal surfaces, raising questions of the immune system's integrity[13]. Alternatively, intrinsic molecular aberrations in

[10] Ferrero, S; Ragni, N; Remorgida, V; *Antiangiogenic therapies in endometriosis. British journal of pharmacology.* 2006, 149(2): 133-135.

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[12] Ferrero, S; Ragni, N; Remorgida, V; *Antiangiogenic therapies in endometriosis. British journal of pharmacology.* 2006, 149(2): 133-135.

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pelvic endometriotic implants were proposed to significantly play a role in the development and progression of endometriosis, by initiating or sustaining inflammatory responses. Secretion of interleukin-6 ( or even IL-8) from endometriotic cells has been proposed to be a driver of endometriosis progression[14]. Aberrant expression of aromatase, certain cytokines such as interleukin-6 present (IL-6) in the peritoneal fluid or even aberrant expressions of tissue metalloproteinases in addition to deficiency of 17-hydroxysteroid dehydrogenase (17- HSD) type 2 as well as resistance to the protective action of progesterone are just a few of the encountered molecular abnormalities[15]. Aromatase is the key enzyme for the conversion of precursor steroids to estrone and estradiol[16], whereas 17-HSD type 2 in endometrium metabolizes the biologically active estrogen termed estradiol to an inactive steroid termed estrone. Since endometriosis is an estradiol-dependent disorder[17], aberrant aromatase expression and 17-HSD type 2 deficiency in this tissue are of paramount importance in its pathophysiology[18].

The morphologic appearance of endometriosis is marked by proliferation, infiltration and severe adhesions around the surrounding tissues, explaining a wide range of clinical manifestations varying from pelvic pain to infertility[19]. Research into its pathogenesis has focused on anatomic, hormonal, immunologic and genetic factors[20], although the entire etiopathology has not been clearly explained yet. Medical treatments for endometriosis are usually aimed at reducing the endogenous steroid production. Medroxyprogesterone acetate, danazol, oral contraceptives and GnRH-a are all effective in the pain-associated symptoms of endometriosis[21] and are also effective in the regression[22] of the endometriotic lesions. However, their adverse effects limit their long-

Ruxandra; Berceanu, Costin; Bratu, Ovidiu; Pituru, Silviu; Onofriescu, Mircea; Matasariu, Daniela Roxana; *The influence of progesterone on immunohystochemical markers in endometriosis*, Rev Chim, 2018, 69 (3): 581-584.

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[17] Bremer, Andrew A; Miller, Walter L; *Regulation of Steroidogenesis*, CellularEndocrinology in Health and Disease, Academic Press, 2014, 207-227, ISBN 9780124081345, https://doi.org/10.1016/B978-0-12-408134-5.00013-5.15; Velazquez, EM; Mendoza, S; Hamer, T; Sosa, F; Glueck, CJ; *Metformin therapy in polycystic ovary syndrome reduces hyperinsulinemia, insulin resistance, hyperandrogenemia, and systolic blood pressure, while facilitating normal menses and pregnancy*, Metabolism, 1994, 43(5): 647-654.

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[20] Brătilă, Elvira; Ionescu, Oana-Maria; Badiu, Dumitru-Cristinel; Berceanu, Costin; Vlădăreanu, Simona; Pop, Doina Mihaela; Mehedinţu, Claudia; *Umbilical hernia masking primary umbilical endometriosis.* Rom J Morphol Embryol, 2016, 57(2): 825-829.

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term use, and recurrence rates after cessation of therapy are high. Additionally, they have no benefit for endometriosis-associated infertility[23]. Therefore, new agents, which present synchronous fertility treatment with improved side-effect profiles, are needed. These treatments should also be as effective as hormonal treatments.

What needs to be taken into consideration are anti-inflammatory agents which may nurture greater therapeutic potential for the disease than actually expected[24].

**THE METFORMIN EFFECT:**

Metformin is one of the most widely used antidiabetic agents improving insulin sensitivity. In reproductive medicine, the drug has been successfully used for the treatment of polycystic ovary syndrome, known to be etiologically determined by insulin resistance[25]. In women with PCOS, metformin treatment restores the cyclic nature of menstruation and increases ovulation, fertilization and pregnancy rates[26]. The supposed mechanism of action of metformin in endometriosis is proposed to be a decrease in aromatase enzyme activity and inhibition of proliferation on endometrial glands[27]. Therefore, circulating estradiol levels are decreased in response to the increase in SHBG levels, thus reducing and affecting the dynamics of endometrial ectopic tissue[28].

Amongst other properties which may be eventually translated, metformin seems to reduce obesity-associated inflammatory status and other inflammatory responses, reducing serum C-reactive protein levels in women with polycystic ovary syndrome[29]. In addition, it has direct effects on steroidogenesis in ovarian granulosa cells and thecal cells.

An antiproliferative effect of metformin has also been demonstrated in leptin-stimulated vascular smooth muscle cells[30]. Abundant aromatase expression and elevated local estrogen levels

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suggesting local estradiol production by the aromatase enzyme have been demonstrated in endometriotic tissues[31], however lacking from disease free endometrium. On the other hand, metformin has been shown to inhibit FSH, insulin-stimulated progesterone and estradiol production in granulosa cells[32]. Thus, metformin may inhibit endometriosis through suppression of both ovarian and local production of estrogens.

**ENDOMETRIOSIS PATHOGENESIS - A CLASSIC:**

Endometriosis is characterized by inflammation, estrogen dependency, and proliferation of endometriotic cells[33]. The main targets we are going to focus on throughout our discussion, of paramount importance shall be steroidogenesis and inflammation.

**A. STEROIDOGENESIS – TARGETING THE AROMATASE ACTIVITY**

Among estrogen-responsive pelvic disorders, aromatase expression was studied in greatest detail in endometriosis. Firstly, extremely high levels of aromatase RNA[34] were found in extraovarian endometriotic implants and endometriomas. Secondly, endometriosis-derived stromal cells in culture incubated with a cAMP analog displayed extraordinarily high levels of aromatase activity comparable to that in placental syncytiotrophoblast[35].

Mullerian tissues are known targets of estrogen action[36]. Until recently, estrogen action has been classically viewed to occur only via an "endocrine" mechanism: in other words, it was thought that only circulating estradiol, whether secreted by the ovary or formed in the adipose tissue, could exert an estrogenic effect after delivery to target tissues via the bloodstream[37]. Several communication manners have been proven to function among aromatase pathways of expression[38].

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[38] Nguyen, L; Chan, SY; Teo, A; *Metformin from mother to unborn child - Are there unwarranted effects?* EBioMedicine, 2018, 35: 394–404. doi:10.1016/j.ebiom.2018.08.047; Li, X; Feng, Y; Lin, JF; Billig, H; Shao, R; *Endometrial progesterone resistance and PCOS*. Journal of biomedical science, 2014, 21(1): 2. doi:10.1186/1423-0127-21-17.

Studies on aromatase expression in breast cancer demonstrated that paracrine mechanisms play an important role in estrogen action in this tissue[39]. Estrogen produced by aromatase activity in breast adipose tissue fibroblasts was demonstrated to promote the growth of adjacent malignant breast epithelial cells[40]. There has also been demonstrated an "intracrine" effect of estrogen in uterine leiomyomas and endometriosis. Disease-free endometrium and myometrium, on the other hand, lack of aromatase expression[41].

Endometriosis should be accepted as an estrogen-dependent condition because it is seen during the reproductive years and generally disappears after menopause[42]. Medical therapy is often aimed at lowering the estrogenic and inflammatory status of endometriosis since they seem to be the main promoters of disease progress.

## B. PROLIFERATION AND INFLAMMATION

Endometriosis is an estrogen-dependent disorder defined as the presence of endometrial tissue outside of the uterine cavity[43]. Although the pathogenesis of endometriosis still remains unclear, the most accepted theory assumes that the endometriotic implants originate by the migration of eutopic endometrial cells through retrograde trans tubal flow into the peritoneal cavity of menstrual debris, with future implantation and growth on an ectopic site[44]. Oxidative stress has been proposed as a potential factor involved in the pathophysiology and ectopic tissue attachment process.

Many factors are important for the degradation of extracellular matrix and the implantation of endometrial tissue in ectopic sites - notably, matrix-metallo-proteinases (MMPs)[45]. It has been shown that blocking MMP activity inhibits the formation of ectopic lesions in experimental models. Vascular endothelial growth factor (VEGF) has been implicated as inducer of attachment,

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proliferation, and neovascularization[46]. Moreover, antiangiogenic agents inhibited the growth of ectopic tissue by disrupting the vascular supply.

Uterine movement is suggested to play roles in various events related to endometriosis[47]. In view of the current concept underscoring the biological implications of mechanical stretch, we speculated that the mechanical stretch exerted by uterine movement might stimulate the production of biochemical mediators like IL-8 in endometrial cells and contribute to inflammation-associating processes, such as menstruation and endometriosis[48].

Endometriosis has a direct effect on adhesion formation. The inflammation associated with endometriosis, through increased levels of peritoneal fluid VEGF, may promote angiogenesis for progressive growth of endometriosis[49].

IL-1β-induced secretion of IL-8 from endometriotic cells has been proposed to be a driver of endometriosis progression. IL-8 levels are increased in the peritoneal fluid of women with endometriosis[50].

The basis for medical treatments of endometriosis is to reduce the endogenous steroid production[51]. However, long-term use of drugs such as progestogens[52] (oral or intrauterine device), danazole, GnRH-analogs, and aromatase inhibitors, is limited by their adverse effects, cost, limited treatment duration, delayed conception, and the high recurrence rates after cessation of therapy. Also, they have no benefit for endometriosis-associated infertility[53].

## HOW DOES METFORMIN ACT AROUND THESE PROCESSES

Metformin is one of the oldest and most widely used oral agents in the treatment of type 2 diabetic subjects, with no effect on insulin secretion[54]. Metformin has not only been shown to reduce the risk for vascular complications but also has protective effects largely independent of its

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well-known antihyperglycemic action[55]. Besides showing antioxidant properties it shows beneficial effects on VEGF and MMPs[56].

IL-1β-induced secretion of IL-8 from endometriotic cells has been proposed to be a driver of endometriosis progression, being increased in the peritoneal fluid of women with endometriosis[57]. Interestingly, metformin has suppressed IL-8 release from human adipose tissue in vitro, and a recent report demonstrated that metformin inhibited IL-1β-induced release of IL-6 and IL-8 in human vascular wall cells[58]. Although we show here that metformin can inhibit IL-1β-induced secretion of IL-8 from ESCs, at the same doses, metformin did not inhibit secretion of IL-8 from eutopic endometrial stromal cells. Thus, metformin seems to exert its anti-inflammatory role by reducing proinflammatory cytokine secretion in specific cell types.

When it comes to endometriosis associated infertility, anti-endometriotic drugs usage may be beneficial for ovulation induction in these cases[59]. Aromatase inhibitors are effective in treating infertility, however displaying hypoestrogenic effects[60]. Metformin therapy for endometriosis may be more beneficial due to the lack of serious side effects reported with the other drugs[61]. Combined with the anti-inflammatory and anti-estrogenic effects of metformin in specific cell types, the direct anti-proliferative effects on stroma cells support its therapeutic potential for endometriosis[62].

The mechanism of action of metformin on endometriosis was proposed to be a decrease in aromatase enzyme activity in the endometriotic implants and suppression of ovarian

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steroidogenesis[63]. Interestingly, metformin has inhibited FSH and insulin-stimulated progesterone and estradiol production in granulosa cells. Metformin may inhibit endometriosis through suppression of both ovarian and local products of estrogens[64].

## RESULTS &PARTICULARITIES OF METFORMIN USE IN ENDOMETRIOSIS

### 1. ISOLATION OF ENDOMETRIAL STROMAL CELLS IN CULTURES

In an in vitro study[65], it was shown that metformin, a biguanide insulin sensitizer, suppresses the inflammatory response, the activation of aromatase enzyme and the proliferation in endometriotic stromal cells after culture in a sterile medium.

a. Isolation and culture of human endometriotic stromal cells (ESCs)[66] has been done in order to obtain a specific measurement of IL-8 needed to assess metformin's effects on proinflammatory status. Preincubation with metformin significantly decreased IL-1β-induced IL-8 production in ESCs in a dose-dependent manner compared with controls. The maximal effect was observed at 1000 μM, but significant decreases were seen at 10 μM[67].
b. In the same culture, aromatase mRNA expression was assessed by real-time quantitative PCR[68]. Metformin decreased cAMP-induced aromatase mRNA levels in ESCs in a dose-dependent manner. The effect of metformin on aromatase activity was evaluated by measuring estrone levels in conditioned media of ESC cultured with androstenedione[69].
c. The effect of metformin on the proliferation of ESCs was examined by measuring incorporation of BrdU into DNA[70]. Measurement of lactate dehydrogenase (LDH) release and trypan blue exclusion test were conducted to examine the effect of metformin treatment on cell viability, as markers of cell proliferation inhibition.

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The present study of Takemura[71] et al demonstrated that metformin suppressed the production of IL-1β-induced IL-8, the activation of aromatase, and the proliferation of ESCs. These effects of metformin support its therapeutical potential in limiting the development of endometriosis.

## 2. HIGHLIGHTING ENDOMETRIOSIS - METFORMIN DYNAMICS IN RAT MODELS

In a rat model with surgically induced endometriosis, Oner et al[72] found that metformin and letrozole caused a statistically significant regression of endometriotic implants impacting levels of SOD, VEGF, TIMP-2, and MMP-9[73], findings which support that metformin treatment reduces the size of endometriotic implants in rats.

## 3. METFORMIN DYNAMICS IN HUMAN MODELS

The prospective study started by including 69 cases subdivided into 34 cases as a control group and 35 cases as a metformin treated group[74]. The patients were divided into the following groups:

1. Group-1 (treated endometriosis group)[75]: consisted of 35 infertile patients diagnosed by diagnostic laparoscopy to have stages 1–2 (minimal-mild, MM) endometriosis. These patients were complaining of one or more symptoms such as dysmenorrhea, pelvic pain, dyspareunia or menorrhagia. The severity of the disease was graded during previous diagnostic laparoscopy according to the revised four-stage scoring system of American Society of Reproductive Medicine (ASRM)[76]. At least 3 months following diagnostic

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laparoscopy, participants were instructed to take oral metformin 500 mg three times daily for 6 months starting with their next menses plus a multivitamin once daily[77].

2. Group-2 (non-treated endometriosis group): consisted of 34 cases with stages1–2 (minimal-mild, MM) endometriosis diagnosed by diagnostic laparoscopy and were complaining of one or more symptoms such as pelvic pain, dyspareunia or menorrhagia. These cases were enrolled in the study at least 3 months after the diagnostic laparoscopy, and received as placebo a multivitamin once per day during the 6 months follow up period[78].

The plasma levels of VEGF, IL-6, and IL-8 were analyzed by using ELISA kits performed according to the methods recommended by the manufacturer, samples were obtained for an estimation of the cytokines levels at the start of the study, in the morning from the fasting patients during the follicular phase (days 5–10) and also at the follow up visits after 3 and 6 months. There was a significant decrease in incidence of symptoms associated with endometriosis such as dysmenorrhea, pelvic pain and dyspareunia[79]. Moreover, and unique to metformin, there seems to be no impedance to ovulation, contrary to other current treatment options do not offer a chance at conception. In the present study, the % of pregnancy after 6 months of metformin therapy was 25.71%, and this was statistically significant ($P < 0.001$) when compared with the % after 3 months therapy, and with the % in the control group after 6 months[80]. The results of our study showed that the levels of IL-6, IL-8 & VEGF were significantly decreased after 6 months of metformin therapy ($P < 0.05$) when compared with the 3 months level[81]. This can be explained by the anti-inflammatory effects of metformin therapy to decrease the inflammatory process associated with the endometriotic implants.

## DISCUSSIONS

Metformin therapy in patients with endometriosis resulted in a significant reduction in the symptomatic cases, increased chance of pregnancy, and a decrease in the levels of serum cytokines, suggesting that it may have a therapeutic potential as an anti-endometriotic drug.

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However, when it comes to other insulin sensitizers such as rosiglitazone and pioglitazone were found in vitro to decrease chemokine and cytokine expression in endometriotic stromal cells, and modulate angiogenesis[82], and also to induce regression of endometriotic lesions[83].

Moravek et al[84] provided a preliminary data about the effectiveness of rosiglitazone, a

insulin sensitizer, in treating endometriosis-related pain in six patients and concluded that it was effective and promising for usage in endometriosis patients desiring the chance to conceive.

## CONCLUSIONS

What we wanted to emphasize throughout this review is that metformin, an easily available drug, facile to use orally, and of minimal cost proves to be a well-tolerated treatment for endometriosis that relieves pain, reduces menstrual disorders, and improves fecundity[85]. The findings of the cited study combined with the data gathered from experiments and endometriosis induction in rats and nonhuman primates, show promise for the potential of using metformin in endometriosis patients desiring the chance to conceive[86]. However, what would need to be thoroughly done in order to properly apply the results we currently have would be to reevaluate and manipulate large numbers of cases supported by diagnostic laparoscopies in the end[87].

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