

**“PER VAGINAM”****TOPICAL USE OF HORMONAL DRUGS IN WOMEN WITH SYMPTOMATIC DEEP  
ENDOMETRIOSIS: A NARRATIVE LITERATURE REVIEW**

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## 24 ABSTRACT

25 **Purpose:** We aim to provide a comprehensive overview of the role of the vagina as a route for drug  
26 delivery and absorption, with a particular focus on the use of vaginal hormonal compounds for the  
27 treatment of deep infiltrating symptomatic endometriosis.

28 **Methods:** A MEDLINE search through PubMed was performed to identify all published studies in  
29 English language on vaginal hormonal treatments for symptomatic endometriosis.

30 **Results:** Main advantages of the vaginal route include avoidance of the hepatic-first pass metabolic  
31 effect, the possibility of using lower therapeutic dosages, and the reduction of side effects compared  
32 with the oral administration. Studies on endometriosis treatment mainly focused on the use of  
33 vaginal danazol ( $n=6$ ) and the contraceptive vaginal ring ( $n=2$ ). One pilot study evaluated the  
34 efficacy of vaginal anastrozole in women with rectovaginal endometriosis. Most investigations  
35 evaluated the vaginal use of hormonal agents in women with deep infiltrating  
36 endometriosis/rectovaginal endometriosis. Overall, a substantial amelioration of pelvic pain  
37 symptoms associated with endometriosis was observed, particularly of dysmenorrhea. A significant  
38 reduction in rectovaginal endometriotic nodule dimensions measured at ultrasound examination was  
39 detected by some but not all authors.

40 **Conclusions:** The vaginal route represents a scarcely explored modality for drug administration.  
41 High local hormonal concentrations might achieve a greater effect on endometriotic lesions  
42 compared with alternative routes. Future studies should focus on the use of the vagina for delivering  
43 target therapies particularly in patients with deeply infiltrating rectovaginal lesions.

44 **KEYWORDS:** Intravaginal administration; vaginal ring; endometriosis; aromatase inhibitors;  
45 danazol; contraceptive vaginal ring

46

## 47 INTRODUCTION

48 In the last decades, technological innovations in drug delivery have led to a wider range of sites for  
49 drug administration. Historically, the oral route represents the most frequently adopted one.

50 However, another scarcely explored way of drug delivery is the vaginal route; although a large  
51 body of evidence proves the ability of this organ to absorb a wide variety of medication [1].

52 The first published reports of drugs administered intravaginally are dated 1918, when Macht  
53 reported the absorption of morphine, potassium iodide and atropine [2]. Since then the vaginal route  
54 has been adopted for numerous, chemically different, compounds, such as misoprostol,  
55 bromocriptine, indomethacin, antimicrobials, and various steroidal hormones including estrogens,  
56 progestogens and androgens [1, 3].

57 An important field of application for intravaginal therapies could be endometriosis; in fact,  
58 in the past years, some authors have evaluated the efficacy of various vaginal hormonal compounds  
59 for the treatment of rectovaginal endometriosis with promising results [4-6].

60 Endometriosis is an estrogen-dependent chronic inflammatory disorder that requires a life-  
61 long management plan. Some authors suggest that women with endometriosis should no longer be  
62 evaluated as a single and unique population [4]. In this optic, each woman should have a tailored  
63 approach based on her main disabling symptom and the type of lesion [4]. In particular, deep  
64 infiltrating endometriosis (DIE) represents the truly severe endometriotic disease [7]. DIE is a form  
65 of endometriosis characteristically related to marked proliferation of smooth muscle cells and  
66 fibrosis [8]. Deep lesions could infiltrate the muscular layer of different hollow organs, such as  
67 vagina, bowel, and bladder. Patients with DIE are usually the ones complaining most for pain and  
68 with the greatest alteration on the quality of life (QoL).

69 Long-term adherence to treatment is pivotal to ensure an effective clinical management  
70 [4,9]. The rationale of the pharmacological therapy for symptomatic endometriosis is the

71 establishment of a hypo-estrogenic milieu, generally achievable through the use of hormonal  
72 therapies such as, for instance, estrogen-progestins and progestins [4]. These compounds could be  
73 administered orally, vaginally, intra-uterine, subcutaneously or intramuscularly [4].

74 In this narrative review, we aimed to provide a broad overview on the role of the vagina as a  
75 route for drug delivery and absorption, with a specific focus on the use of vaginal hormonal  
76 compounds for the treatment of deep infiltrating symptomatic endometriosis.

## 77 MATERIALS AND METHODS

78 For this review, the best quality evidence was selected with preference given to the most  
79 recent and definitive original articles and reviews. Information was identified by searches of  
80 Pubmed/MEDLINE and references from relevant articles, using combinations of MESH terms  
81 “intravaginal administration”, “pharmacodynamics”, “endometriosis”, “deep infiltrating  
82 endometriosis”, “vaginal danazol”, “contraceptive vaginal ring”, “vaginal ring”, and “aromatase  
83 inhibitors”. The search was limited to peer-reviewed, full-text articles in the English language. For  
84 most issues, papers published between March 1990 and December 2016 were considered. No  
85 attempt was made to find unpublished studies. Since only published data were considered, the  
86 current research project was exempt from Institutional Review Board approval.

## 87 RESULTS

### 88 ➤ *Role of the vagina as a route for drug delivery and absorption*

89 The vagina is a collapsed organ, in which the surface absorption area is augmented by the presence  
90 of numerous rugae and could reach a maximum of 95 cm<sup>2</sup> in standard conditions [10].

91 One of the peculiar aspects of the vagina is represented by its vascular supply that comprises  
92 a complex network of veins. In particular, the different portions of the vagina are drained by various  
93 venous systems influencing drug absorption depending on the level at which the compound has

94 been introduced [10]. The uterine and ovarian plexus are linked to the venous return of the superior  
95 part of the vagina, and they drain directly into the internal iliac vein, by-passing the hepatic portal  
96 system. In particular, a pharmacological compound administered in the superior part of the vagina  
97 has a specific affinity for the uterine tissues, especially for the endometrium, due to the extensive  
98 vascular connections between these two organs. This phenomenon, given its similarity with the  
99 hepatic-first pass metabolism secondary to the oral administration, has been termed “uterine first-  
100 pass effect” [11]. Instead, the inferior part of the vagina is connected to the hemorrhoidal and  
101 pudenda internal plexus, which leads to the portal system and is subject to the metabolizing action  
102 of the liver [3].

103 In general, drug absorption is a passive process regulated by different factors such as  
104 molecular weight, liposolubility, constancy of diffusion, time, and surface of diffusion [10]. In  
105 addition, vaginal drug absorption is also influenced by some physiological factors, including age,  
106 pregnancy, hormone status, and pH changes. The modifications of this latter element are secondary  
107 to numerous variables such as bacterial colonization, semen, menstruation, and estrogen status.  
108 Vaginal absorption of a drug could be impacted by the presence of a larger volume of vaginal fluids  
109 that can favor a more rapid and efficient dissolution of compounds characterized by low  
110 hydrosolubility but, at the same time, can raise the possibilities of a drug to be ejected due to  
111 gravity. Moreover, the presence of cervical mucus with high viscosity could represent an obstacle to  
112 drug absorption [12].

113 Another factor to keep in mind when prescribing intravaginal therapies is the age of the  
114 patient; in fact, in post-menopausal women, the thickness of the vaginal walls is reduced, and the  
115 absorption of steroids is higher than in fertile women [13]. In addition, changes in hormone levels,  
116 especially estrogen, during the menstrual cycle, cause alterations in the thickness of the epithelial  
117 cell layer, width of intercellular channels, pH, and secretions, with subsequent variations in vaginal  
118 drug absorption [10,14]. Estrogenization of the vaginal mucosa improves absorption of hormones

119 through the vaginal wall [10,15]. Finally, the formulation and the carrier also influence the  
120 absorption rate. For example, creams' absorption is higher compared to rings and tablets [10].

121 One of the most studied vaginal pharmacological compounds is represented by the vaginal  
122 contraceptive ring (CVR) (ethinyl estradiol (EE) 15 µg + etonogestrel (ENG) 120 µg). Numerous  
123 studies have compared the pharmacokinetics of the steroids released by the CVR with those  
124 discharged by various combined oral contraceptive (COC) [16-18]. Timmer and Mulders [17]  
125 performed a randomized crossover study on 16 healthy women demonstrating that maximal serum  
126 concentration ( $C_{max}$ ) of ENG and EE obtained with the vaginal contraceptive ring were 40% and  
127 30% inferior of those gained with a COC containing 150 µg of desogestrel (DSG) and 30 µg of EE.  
128 In the same study group, absolute bioavailability was comparable for EE but higher for ENG with  
129 the CVR compared with the COC (103% vs 79%) [17].

130 A randomized open-label study [18], performed on 24 women, compared different serum EE  
131 levels subsequent to the use of the CVR, of the transdermal patch or of a COC (EE 30 µg +  
132 levonorgestrel (LNG) 150 µg).  $C_{max}$  of EE for the ring, the patch, and the COC were 37.1 pg/ml,  
133 105 pg/ml, and 168 pg/ml, respectively. In addition, analysis of area under the EE concentration-  
134 versus-time curve (AUC) during 21 days of treatment showed that exposure to EE in the CVR  
135 group was 3.4 times lower than in the patch group and 2.1 times lower than in the COC group.  
136 These findings suggest that suppression of ovulation with the CVR is comparable to that reached  
137 with COCs but with lower circulating levels of EE.

138 Moreover, Roumen *et al.* [19] compared the uterine concentrations of EE and ENG after use  
139 of CVR and a COC (EE 20 µg + DSG 150 µg). In both groups, concentration of ENG and EE were  
140 comparable in uterine samples of the myometrium and cervical region. However, unexpectedly, in  
141 women treated with the CVR concentration of both ENG and EE were significantly lower in tissue  
142 samples from the endometrium. Finally, Dogterom *et al.* [20] performed a pharmacokinetic study in  
143 order to assess the potential interaction of a concomitant treatment with oral antibiotics (amoxicillin

144 and doxycycline). No differences in ENG or EE serum concentrations were identified between  
145 women using vaginal contraceptive ring alone versus those receiving the ring plus either of the  
146 antibiotics. Conversely, co-administration of vaginal anti-mycotic resulted in a slight rise in  
147 systemic exposure of both ENG and EE, in particular with suppositories antifungal formulations  
148 [21].

149 Pharmacokinetic studies on progestogen only CVR demonstrated a good correlation  
150 between *in vitro* and *in vivo* release rates of LNG [22]. Serum levels reached the peak concentration  
151 2 hours after the insertion of the ring, after which levels diminished at a rate of 0.2%/day during 90  
152 days of continuous use. Other pharmacokinetic studies on various progestogen-releasing vaginal  
153 ring have been conducted. As an example, Landgren *et al.* [23, 24] evaluated two types of vaginal  
154 ring releasing norethisterone (NET) at a rate of 50 µg/daily and 200 µg /daily. The ring containing  
155 the lower dose of NET did not inhibit ovulation with consequent high pregnancy rate, whereas  
156 those releasing the higher dose of NET displayed a strong ovulation-inhibiting effect but showed a  
157 high frequency of unscheduled bleeding.

158 ➤ *Vaginal therapies for the treatment of symptomatic deep infiltrating endometriosis*

159 The intravaginal route has been underused for the treatment of endometriosis (Table 1). The  
160 majority of the evidence regarding the vaginal route for the management of endometriosis are  
161 derived from the use of danazol.

162 Danazol is a synthetic derivative of 17 $\alpha$ -ethyniltestosterone with mild androgenic activity.  
163 Oral danazol has been widely adopted in the treatment of endometriosis at the daily dosage of 400-  
164 800 mg, resulting in high serum concentration of the compound, which may elicit androgenic  
165 adverse effects, such as acne, hirsutism, weight gain, deepening of voice pitch, and alteration of the  
166 blood lipid profile [32,33]. Oral danazol acts on endometriotic lesions at two levels: firstly, danazol  
167 shows inhibitory effect on the hypothalamic-pituitary-ovarian axis; secondly, danazol can work



168 directly on endometriotic tissues through the inhibition of aromatase activity, reducing  
169 inflammation and the production of angiogenic factors, making endometriotic lesions inactive and  
170 atrophic [34]. However, danazol used orally for an extended period is not advisable, due to the  
171 important androgenic side effects, and for this reason different study groups have assessed its  
172 vaginal use (Table 1).

173 First, Igarashi *et al.* [25,26] evaluated the efficacy of a danazol-loaded vaginal ring in  
174 women with endometriosis. In the first study [25], the vaginal ring (releasing 95 mg of danazol per  
175 day) was used in 35 infertile women with endometriosis. Authors found a substantial amelioration  
176 in both dysmenorrhea and a decrease in the extent of pelvic endometriosis. In addition, as vaginal  
177 danazol did not inhibit ovulation, 13 patients conceived while using the vaginal ring. This point is  
178 particular concerning due to the potential teratogenic effects of this drug [35]. In fact, as reported in  
179 a previous retrospective review [35] on 129 women exposed to danazol during pregnancy, only 37  
180 delivered a normal male and 24 a non-virilized female, whereas, 23 women gave birth to a virilized  
181 female. All the abnormalities have been reported in those patients who continued danazol  
182 administration after the 8<sup>th</sup> week of gestation. In this view, danazol should remain contraindicated in  
183 pregnancy and a careful contraceptive advice to patients under danazol therapy should be given.

184 In the second study [26], danazol was administered using a vaginal ring drug delivery  
185 system containing 1500 mg of danazol. Igarashi *et al.* [26] enrolled 56 infertile women with  
186 endometriosis, 42 with DIE, and 14 with ovarian endometriomas. All the enrolled patients showed  
187 normal menstruation pattern and basal body temperature curves; in addition, 39 of them conceived  
188 during the study period, and none of the female infants born presented signs of masculinization.  
189 Serum levels of danazol remained undetectable. The effectiveness on pain symptoms differed in the  
190 two groups, in fact, dysmenorrhea disappeared in 76% (32/42) of the patients with DIE, but only in  
191 50% (7/14) in the group with ovarian endometriomas. In addition, also at transvaginal ultrasound  
192 the size of the ovarian cysts, conversely to endometriotic deep nodules, remained unchanged in

193 almost 80% of cases (11/14). Moreover, one woman out of four (2/8) conceived in the ovarian  
194 endometriotic cyst group, compared to one out of two (17/31) in the DIE group. A plausible  
195 explanation for this different outcome in the two study groups could be attributed to the proximity  
196 of deep endometriotic lesions to the site of action of the vaginal ring. In this way, the drug released  
197 from the vaginal ring should ideally reach higher concentrations in the vaginal endometriotic lesion.

198 Razzi *et al.* [27] treated 21 symptomatic patients with DIE with low dose vaginal danazol  
199 (200 mg/d) for 12 months. Dysmenorrhea and dyspareunia were relieved in 19 out of the 21 women  
200 and were improved in the remainders. Relief from dyschezia was also observed. At ultrasound  
201 examination, a reduction of the volume of the rectovaginal nodule was demonstrated (from  $3.1 \pm 1.2$   
202 mL to  $1.2 \pm 0.8$  mL). Moreover, the vaginal use did not alter metabolic or thrombophilic parameters,  
203 and the main reported side effect was a vaginal irritation during the first month of treatment in only  
204 four cases. These promising results were similar to those obtained by Bhattacharya *et al.* [29], who  
205 adopted in 21 patients with severe endometriosis (stage IV), for a total treatment period of six  
206 months, the same vaginal dosage of danazol.

207 In 2011, Ferrero *et al.* [30], evaluated the effectiveness of therapy with very low-dose of  
208 vaginal danazol (100 mg/d) in patients with rectovaginal endometriosis and persistent pain  
209 symptoms refractory to the use of a levonorgestrel-releasing intrauterine device (LNG-IUD). 15  
210 women were enrolled for the study, and the daily administration of danazol lasted six months. At  
211 the end of the study period, the satisfaction rate associated with the treatment was 80%. In addition,  
212 the volume of the rectovaginal plaque decreased during treatment (from  $2.3 \pm 0.9$  cm<sup>3</sup> to  $1.7 \pm 0.8$   
213 cm<sup>3</sup>). Side effects were minimal and well-tolerated, the most frequently reported was acne ( $n = 4$ ).

214 The above-mentioned studies confirm the potential beneficial role of vaginal danazol in the  
215 treatment of women with endometriosis, in particular in those with deep infiltrating and vaginal  
216 localizations. Contrarily to oral administration, vaginally administered danazol showed limited  
217 androgenic side effects, and its serum levels remained low or undetectable. These results are

218 consistent with those of Mizutani *et al.* [36], who demonstrated that danazol concentration in the  
219 ovary and uterus after daily vaginal administration of 100 mg of danazol were analogous to those  
220 reached after oral administration of 400 mg, and, at the same time, serum level after daily  
221 intravaginal danazol use was less than 1/20 of that after oral administration.

222 Another vaginally administered drug that has been evaluated for the treatment of  
223 endometriosis is an estrogen-progestogen contraceptive ring [5,31] (Table 1). In 2010, Vercellini *et*  
224 *al.* [5], performed a patient preference trial on 207 women with recurrent moderate or severe pelvic  
225 pain after conservative surgery for symptomatic endometriosis, comparing the CVR (EE 15 µg +  
226 ENG 120 µg) and a transdermal patch (EE 20 µg + norelgestromin 150 µg). A total of 123 (59%)  
227 women preferred the CVR, whereas 84 (41%) chose the patch. Both treatments were administered  
228 continuously for 12 months. Fifty-nine (28%) patients with rectovaginal endometriosis were  
229 included in the study group. The rate of withdrawal was high in both group, 36% in the CVR group  
230 and 61% in the transdermal patch group. Bleeding control was suboptimal with both delivery  
231 systems, in fact, by the end of the study period 46% of the patients who chose the ring and 42% of  
232 those who preferred the patch changed from continuous to cyclic use. Pelvic pain symptoms were  
233 reduced in both groups. In particular, the CVR performed better than the patch regarding  
234 dysmenorrhea relief in patients with rectovaginal endometriotic lesions. A considerable  
235 amelioration of deep dyspareunia was also obtained. No significant major adverse event was  
236 recorded. At the end of study, 71% of the patients who have chosen the CVR declared to be  
237 satisfied with the treatment, whereas the percentage of satisfied women dropped to 48% in the patch  
238 group. In the sub-group of women with rectovaginal lesions, the percentage of satisfied women was  
239 higher in both groups: 79% in the CVR group and 57% in patients treated with the patch.

240 A second patient preference trial [31] compared the CVR, administered cyclically, to the  
241 desogestrel-only contraceptive pill (75 µg/d) for the treatment of symptomatic patients with  
242 rectovaginal endometriosis. The duration of the treatment was 12 months; 60 women chose the

243 progestin-only pill and 83 the CVR. At the end of the study, the rate of satisfied women was higher  
244 in the group treated with desogestrel-only pill (61.7% vs. 36.1%). The discontinuation rate and the  
245 reduction in volume of rectovaginal nodules were similar in the two study groups. Gastrointestinal  
246 symptoms, chronic pelvic pain and deep dyspareunia were improved more in the progestin-only pill  
247 than in CVR group.

248         The potential beneficial role of vaginally administered aromatase inhibitors has been  
249 evaluated in a pilot study [6] on ten symptomatic patients with histologically confirmed  
250 rectovaginal endometriosis. Women received 0.25 mg/d of vaginal anastrozole for 6 months. The  
251 preliminary results were encouraging and patients reported an improvement of dysmenorrhea and  
252 QoL. However, chronic pelvic pain, dyspareunia as well as rectovaginal lesion size remained  
253 unchanged. The dual energy absorptiometry (DEXA) scans, performed before the initiation of the  
254 study and within one month after the end of the treatment, show no change in bone mineral density.  
255 Serum hormonal levels were repeatedly measured during the study period and within one month  
256 after the completion of the treatment. No statistically significant differences were observed in  
257 values for gonadotropins FSH and LH or for P and E<sub>2</sub>. In women with endometriosis the inhibition  
258 of the hypothalamic-pituitary-ovarian axis is of fundamental importance. Therefore, as suggested by  
259 Hefler *et al.* [6], a combined therapy with a hormonal drug capable of inhibit ovulation should be  
260 proposed.

261         Recently, a vaginal ring containing a combination of anastrozole (ATZ) and the progestin  
262 LNG has been developed for the treatment of endometriosis and tested in healthy cycling female  
263 cynomolgus monkeys [37]. The intravaginal system was effective in causing a reduction of  
264 systemic E<sub>2</sub> by about 30% in the proliferative phase without stimulating the development of ovarian  
265 cysts or the increase of FSH. In fact, one of the major limitation of aromatase inhibitors use in  
266 premenopausal women is the possible stimulation of follicular development, secondary to the rising  
267 of gonadotropin levels, which can lead to the formation of ovarian cysts [38]. To prevent this

268 phenomenon, a combination of aromatase inhibitors and a combined oral contraceptive (OC) or  
269 progestin could be advisable.

270 A multicenter Phase I, randomized controlled trial [39], was conducted to evaluate the  
271 pharmacokinetics, pharmacodynamics, safety and tolerability of intravaginal ring containing three  
272 different dose combinations of AZT and LNG (Treatment A: 1 ring, 500  $\mu\text{g}/\text{d}$  ATZ and 20  $\mu\text{g}/\text{d}$   
273 LNG; Treatment B: 1 ring, 1000  $\mu\text{g}/\text{d}$  ATZ and 30  $\mu\text{g}/\text{d}$  LNG; Treatment C: 2 ring, 1500  $\mu\text{g}/\text{d}$  ATZ  
274 and 40  $\mu\text{g}/\text{d}$  LNG. The trial was performed on 60 healthy premenopausal women and the treatment  
275 period consisted of 56 days (two cycles of 28 days without ring-free interval). During the study  
276 period the mean size of the largest follicle-like structures was higher in all three treatment arms than  
277 during the pre-treatment cycle; however, changes in the mean size of the cysts were comparable to  
278 those described for low-dose progestin-only OC and generally resolved during the 2-month  
279 treatment period. Serum E2 levels were below 20 pg/ml in both cycles only in the mid- and high-  
280 dose groups. All the three combinations of AZT and LNG were well tolerated. To achieve a LNG  
281 systemic exposure similar to that obtained after daily oral administration, the optimal intravaginal  
282 ring LNG delivery rate was 40 pg/ml. The doses selected for AZT to be investigated in Phase 2  
283 studies on patients with endometriosis were 300, 600 and 1050  $\mu\text{g}/\text{d}$ .

## 284 DISCUSSION

285 The main potential advantages of the vaginal administration of therapeutics are the reduction of  
286 daily dosages and the continuity of drug release. Moreover, the possibility of extending the interval  
287 between doses represents a favorable option for the patient that can enhance her adherence to the  
288 drug regimen [3].

289 Another advantage of the vaginal route compared to oral administration is the by-passing of  
290 gastrointestinal absorption and thus of the hepatic first-pass effect. Unpredictable factors, like  
291 vomiting or reduced absorbent capacity of the bowel, could interfere with the gastrointestinal  
292 absorption. In addition, both the liver and the gastrointestinal system are accountable for the

293 elimination of numerous compounds [40]. For this reason, avoidance of the hepatic first-pass effect  
294 is especially useful for drugs subject to an intense hepatic metabolism. As an example, natural  
295 estrogens, when given orally, are metabolized by the liver for the 95%. Consequently, the  
296 possibility of vaginal drug delivery permits the prescription of lower doses with reduced incidence  
297 of side effects and, at the same time, is able to reach the same pharmacodynamic effect [41]. In fact,  
298 the avoidance of hepatic first pass metabolism with vaginal delivery of estradiol permits the use of a  
299 10- to 20-fold lower dose to obtain the same systemic levels compared with oral administration  
300 [41].

301 An additional advantage of the intravaginal route is its reversibility and easiness of use,  
302 which makes the woman in control of its application. However, at the same time, this represents one  
303 of the major obstacles to overcome. In fact, a large part of the female population perceives the idea  
304 of inserting a drug (ring, tablet or gel) in the vagina as a “foreign body” that can interfere with  
305 personal hygiene or can cause adverse effects on coitus [42]. In addition, as demonstrated by an  
306 online survey in 2004, entitled the International Vagina Dialogue Survey, more than half of the  
307 interviewed didn't know the correct anatomy of the vagina and only 35% were aware of the  
308 possibility of using the vaginal route for drug administration. In this view, the role of gynecologists,  
309 in counseling their patients regarding popular misconceptions about the vagina and the applicability  
310 of this route for drug administration is of primary importance [42].

311 The disadvantages associated with the intravaginal route include the risk of spontaneous  
312 expulsion of vaginal rings, that occasionally goes unnoticed, the possibility of increased local  
313 adverse effects, such as vaginal infection, increased leucorrhea, vaginal discomfort and local  
314 lesions. In a large observational study on the use of the CVR [43] the most commonly reported side  
315 effects were headache (6%), vaginitis (6%) and leucorrhea (5%). Vaginal discomfort and ring-  
316 related local events were described in 2% and 4% of the enrolled patients [43]. The withdrawal rate  
317 due to vaginitis and leucorrhea was low (1.3%) [43]. Another clinical trial [44] compared the CVR

318 with a COC (EE 30 µg + LNG 150 µg), with a follow-up of 12 months. A high percentage of  
319 women enrolled in the CVR group reported vaginitis and leucorrhea during the study period (11%),  
320 however, only 1% discontinued the CVR for this reason. Fine *et al.* [45] evaluated the safety and  
321 efficacy of the CVR in 81 women who had undertaken a surgical abortion. The CVR was inserted a  
322 week after the surgical procedure. After one month, 4% of the patients had experienced a bacterial  
323 vaginosis and 2% a Candida infections. Finally, the increased risk of bacterial vaginosis was  
324 supposed also by Archer *et al.* [46], who reported an improved Nugent score in 40% of vaginal  
325 contraceptive rings users.

326 Another field of concern regarding CVR is the fear of feeling the ring during coitus and  
327 during everyday activity. Two large studies [47-48] showed reassuring results, in fact, more than  
328 85% of the participants reported that they did not perceive the ring during sexual intercourses.

329 The rationale behind the use of local treatments for vaginal endometriosis includes the  
330 above-mentioned advantages of the vaginal route, comprising the avoiding of the hepatic first-pass  
331 effect, the possibility of adopting lower doses than those required for oral administration, the  
332 reduction of side effects. Moreover, a local administration in close proximity to the endometriotic  
333 nodules and plaques could result in higher concentrations of the drug in the surrounding area, with  
334 the potential result of a “target lesion” therapy. Overall, a substantial amelioration of pelvic pain  
335 symptoms associated with endometriosis was observed, particularly of dysmenorrhea.

336 Several studies have demonstrated that vaginal administration of danazol allows the use of  
337 significantly lower doses than those adopted for the oral route, with serum concentration being  
338 lower than after oral assumption [34, 36]. In fact, low-dose vaginal danazol has been adopted with  
339 positive results in mild-to-moderate endometriosis at a daily dose of 100 mg and 200 mg [27,29,30]  
340 (Table 1), whereas, in most studies, higher oral daily doses (400 to 800 mg) are needed to achieve  
341 positive outcomes on pain symptoms [49-62]. In addition, vaginal danazol has been proven to be  
342 effective for endometriosis-related pain with limited side effects [25-30,34].

343 In addition, an increased expression of aromatase activity has been demonstrated in  
344 endometriosis lesions. This overexpression provokes a hyperestrogenic milieu within the implant  
345 that could favor the progression of the disease [34]. Furthermore, aromatase activity is absent in  
346 normal human endometrium and is increased in endometriosis lesions [63]. Almost all the available  
347 evidence concerning the use of aromatase inhibitors in patients with endometriosis refer to oral  
348 drugs [64-74]. Only a pilot study [6] has evaluated the potential role of vaginal anastrozole on  
349 women with symptomatic rectovaginal endometriosis, with encouraging preliminary results. Given  
350 this background, the use of vaginal drugs with inhibitory activity on this enzyme, like danazol or  
351 aromatase inhibitors, could have a role specifically in the treatment of vaginal endometriosis.

## 352 CONCLUSIONS

353 Future studies should focus on implementation of the use of the vagina as a drug delivery  
354 modality, in particular in those patients with deep infiltrating and vaginal lesions. In fact, as  
355 demonstrated in previous studies [5,26], vaginal treatments appeared efficacious mostly in women  
356 with rectovaginal lesions, probably due to the higher local concentration of drug obtainable from  
357 direct contact between the drug itself and the lesions located in the posterior fornix.

358 Women should be carefully instructed about the correct modality for positioning drugs  
359 intravaginally. In fact, the compounds should be placed at bedtime, deeply into the cranial portion  
360 of the vaginal canal to prevent drug dispersion with subsequent variability of serum levels.  
361 Moreover, the correct placement of vaginal drugs appears crucial particularly in women with DIE,  
362 in order to obtain a high drug concentration near the endometriotic vaginal lesions and avoid  
363 absorption of the compound into the hemorrhoidal and internal pudendal vascular plexuses.

364 Ideally, in endometriosis patients' hormonal drugs should inhibit ovulation. Therefore, in  
365 case aromatase inhibitors are used vaginally, they should be combined with progestins at doses  
366 sufficient to inhibit the hypothalamic-pituitary-ovarian axis.



367           The vaginal route represents a partially unexplored route for drug administration, especially  
368 in women with vaginal endometriosis. Transvaginal drug delivery offers several biochemical and  
369 metabolic advantages, beyond its simplicity and reversibility of use. There is a great need for  
370 further research in this promising field of application of hormonal drugs for the treatment of the  
371 most demanding forms of endometriosis.

372 **CONTRIBUTION TO AUTHORSHIP**

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