

Estrogen and Pelvic Organ Prolapse

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Abstract

Pelvic organ prolapse (POP) is a multifactorial disease with a complex and largely unknown etiology and pathophysiology. Hypoestrogenemia may be one of the risk factors associated with POP. Recent studies suggest a potential role of estrogen and its receptors in the pathogenesis of POP. Here, we summarize current research regarding the relationship between estrogen and POP to establish a theoretical foundation for using estrogen in POP treatment. Estrogen plays an important role in collagen and elastin metabolism of connective tissues through down-regulating matrix metalloproteinases and increasing cystatin C expression. However, previous studies have shown contradictory data regarding estrogen receptor expression in patients with POP compared to non-POP controls. At this time, there is no conclusive evidence suggesting a causal role of estrogen in POP. Further well-designed studies are necessary to illuminate both the molecular mechanisms of estrogen function and the role of estrogen in POP.

Keywords: Pelvic organ prolapse; Estrogen; Estrogen receptor; Collagen; Matrix metalloproteinases

Introduction

Pelvic organ prolapse (POP) refers to the abnormal descent or herniation of pelvic organs from their normal attachment sites or their normal positions and can involve the bladder, uterus, vagina, small bowel, and rectum. Although only 10-20% POP patients seek care, POP affects half of all women [1]. Clinical presentation of POP can be variable, including lower back pain, painful intercourse, a feeling that something is falling out of the vagina, urinary leakage, a chronic urge to urinate, constipation, and vaginal spotting or bleeding. Even if not life-threatening, POP can significantly impact a woman's quality of life and cause disability. Wu et al. estimated that the risk of requiring surgical correction for POP by the age of 80 is 12.6 % and that 9.2 million women in United States will be affected by this condition by the year 2050 [2,3].

Contributing risk factors to the development of POP include obstetric causes such as vaginal delivery, parity and the delivery of large infants. Non-obstetric risk factors such as increasing body mass index, aging, race, chronically increased intra-abdominal pressure, and genetic factors have also been suggested. Since there is a direct correlation between the incidence of POP and aging, and circulating estrogen levels are drastically decreased in postmenopausal women, hypoestrogenemia may be a causative factor for the development of POP. Current studies have focused on the relationship between estrogen and POP to ascertain a theoretical basis for the therapeutic use of estrogen in POP.

Pelvic organs are supported by pelvic floor muscles, connective tissues, and pelvic ligaments. Dysfunction of any of these structures will reduce the support of pelvic organs, causing organs to descend

onto the vagina and eventually leading to POP. Interstitial collagen, elastin, and fibroblasts are important structural constituents of pelvic connective tissues [4]. Therefore, understanding the role of estrogen in the metabolism of these components may give us new insight into the pathogenesis of POP.

Estrogen and collagen

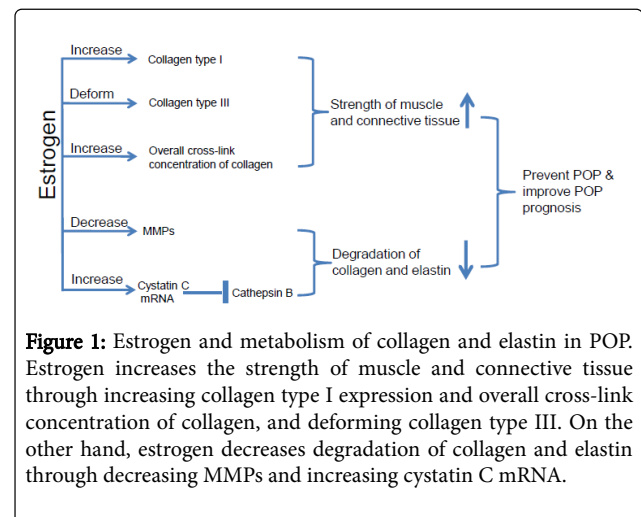


Figure 1: Estrogen and metabolism of collagen and elastin in POP. Estrogen increases the strength of muscle and connective tissue through increasing collagen type I expression and overall cross-link concentration of collagen, and deforming collagen type III. On the other hand, estrogen decreases degradation of collagen and elastin through decreasing MMPs and increasing cystatin C mRNA.

Estrogen has a profound influence on pelvic collagen synthesis. Collagen is one of the main constituents of connective tissue in the pelvis. Type I collagen is present in organized fibers and ligamentous tissue, whereas type III collagen is commonly found in loose areolar tissue. The composition of collagen subtypes differs between women

with POP and controls. In general, expression of type III collagen, which provides weaker structural support than type I collagen, is higher in the ligaments of prolapsed uteri in comparison with ligaments of normal uteri, leading to decreased strength in the pelvic supportive ligaments [5]. Estrogen can increase type I collagen expression and deform type III collagen in fibroblasts, leading to increased muscular strength [6]. Muscle strength can also be improved by enhancing type I collagen expression in pelvic organ tissues [6]. Additionally, estrogen can also increase overall collagen cross-linking and replace aged collagen with new tissue, leading to increased tissue strength (Figure 1) [7].

Matrix collagen in connective tissue is generally degraded by matrix metalloproteinases (MMPs), which are a family of zinc-dependent endopeptidases [8-11]. MMP-1 and MMP-9 both cleave denatured collagen chains. MMP-2 can act on both intact and denatured collagens. Cathepsin B cleaves the cross-link sites of collagen during the first step of collagen degradation [12]. MMP-13 degrades fibrillar collagens and activates other MMPs to initiate degradation pathways (Figure 2) [13].

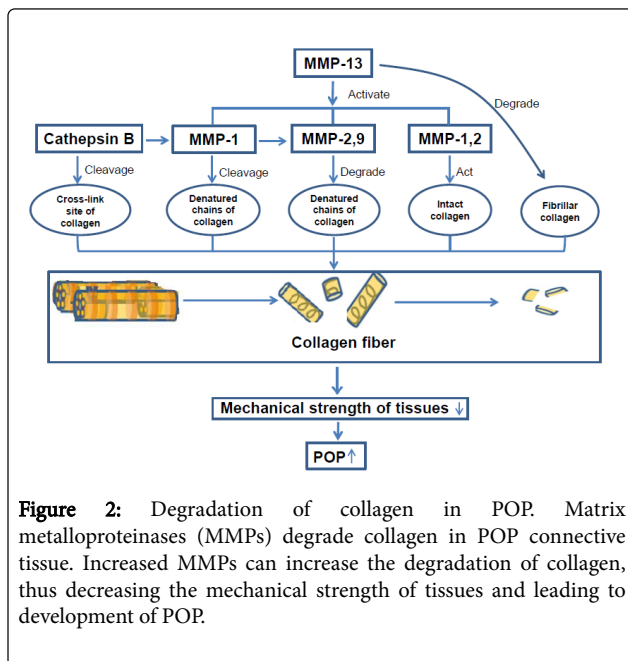


Figure 2: Degradation of collagen in POP. Matrix metalloproteinases (MMPs) degrade collagen in POP connective tissue. Increased MMPs can increase the degradation of collagen, thus decreasing the mechanical strength of tissues and leading to development of POP.

Estrogen can down-regulate MMP-9 and MMP-12 in the vaginal wall, leading to decreased collagen degradation [14]. Additionally, estrogen decreases MMP-13 proteolytic activity by activating MMP-13 fragmentation in the cell-associated ubiquitin-proteasome pathway, leading to an overall decrease in MMP-13 activity [15]. Estrogen has been shown to increase cystatin C, an inhibitor of the protease cathepsin, in fibroblasts and smooth muscle cells of the rhesus macaque vagina [16]. Thus, estrogen inhibits collagen degradation via down-regulation of MMPs (Figure 1).

Estrogen and elastin

Elastin is also one of the main constituents of connective tissue in the pelvis. In contrast to the strength provided by collagen, elastin promotes tissue flexibility, extension, resilience, and recoil [4]. Connective tissue derived from the uterosacral ligament and vaginal

wall of women with POP has been found to express lower levels of elastin [17,18].

Lysyl oxidase (LOX) is a copper-dependent amine oxidase. The synthesis of mature elastin fibers requires LOX-Like 1 (LOXL1). The expression of LOXL1 mRNA in POP compared to non-POP tissues is controversial. Some studies have shown that LOXL1 mRNA expression levels in POP tissues are higher than non-POP tissues [19,20], while other studies have demonstrated opposite results [21,22]. However, after treatment with estradiol (E2), the expression of LOX family genes increases in the vagina of mice. These results indicate that E2 treatment could increase expression of LOX genes and suggest that E2 may be used in interventions for POP in humans [23]. Overall, the specific mechanisms of LOXL1 in the development of POP are complex and remain unclear. Further studies are necessary to elucidate the role of LOXL1 in development of POP.

Neutrophil elastase (NE) and MMP are the most important proteases involved in the proteolytic degradation of elastin [24]. Elastolytic protease activities of NE, MMP-2, and MMP-9 are increased in uterosacral ligaments of women with POP [25]. Additionally, immature elastin, mature elastin, and the elastin-degrading enzyme MMP-9 are increased in vaginal wall tissues in women with POP as compared to controls [26]. Several studies have demonstrated that estrogen can down-regulate MMPs in the vaginal wall and possibly decrease elastin degradation, suggesting a role of estrogen in elastin metabolism (Figure 1).

Estrogen and fibroblasts

Pelvic connective tissues consist of a small number of fibroblasts surrounded by an extracellular matrix (ECM). Fibroblasts remodel their surrounding matrix in response to mechanical and biochemical stimuli, which maintains the strength of the ECM. Fibroblasts derived from POP tissues respond differently to mechanical and biochemical stimuli as compared to fibroblasts from controls. Fibroblasts from POP tissues have lower contractile capacities and delayed mechano-responses compared with non-POP site cells [27]. Furthermore, POP fibroblasts produce stiffer matrices than cells from non-prolapsed tissues. Therefore, prolapsed tissues have increased stiffness and decreased connective tissue strength, which may contribute to the development of POP [27,28].

Estrogen also affects the cytoskeleton and cell morphology of fibroblasts. Estrogen therapy can suppress excessive and abnormal remodeling of fibroblasts and their cytoskeletons, thereby improving the mechanical properties of connective tissues. Thus, estrogen may maintain the integrity and function of fibroblasts and connective tissues during mechanical stretching to prevent POP and improve POP prognosis [29].

Estrogen receptors

Estrogen function is primarily mediated via specific intracellular estrogen receptors (ERs), which are members of a super-family of ligand-activated transcription factors expressed in target tissues [30]. There are two major ERs, ER α [31,32] and ER β [33,34], and estrogen binds to both ERs with high affinity and specificity. The expression of ER α and ER β is different in various target tissues. ER α is expressed at high levels in the uterus. In contrast, other estrogen target tissues such as the prostate [35], salivary glands [36], testis [37], ovary [38], vascular endothelium [39], smooth muscle [40], and immune system [41] have high expression of ER β . Estrogen and ER α agonists induce

increased collagen bundle thickening and fibronectin deposition in human skin [42]. ER β is involved in elastin and collagen homeostasis either through transcriptional effects on elastin and collagen genes or through regulation of proteases involved in the degradation of these proteins [43]. The rapid non-genomic signaling pathways mediated by cell membrane-associated ERs have also been recognized. Various functions of ER α and ER β require not only receptor-ligand binding, but also activation of downstream modulators of gene expression [44].

Estrogen induces polyphenic responses in target tissues through classic steroid receptor-mediated pathways and transactivation of Fos/Jun (AP1) mechanisms in women with POP. The specific receptor subtypes may mediate different functions of estrogen [45]. As previously mentioned, hypoestrogenemia is associated with POP, and estrogen plays an important role in collagen and elastin metabolism in connective tissues. However, ER expression levels in POP and non-POP tissues are controversial. The distribution and function of different ERs in the pelvic floor connective tissue are elaborated below.

Grigoriadis et al. demonstrated that the expressions of both ER α and ER β are significantly reduced in the pubocervical fascia tissues of postmenopausal women diagnosed with POP as compared with non-POP controls [46]. Shi et al. found that levels of ER α protein are 2.2-fold lower in the cervical portion of uterosacral ligaments of POP patients compared with non-POP controls. This may be mediated by post-translational control of ER α expression via increased expression of miR-221 and miR-222 [47]. Consistent with this finding, Other studies also found that the expression of ER α is significantly lower in the uterosacral ligaments of the POP patient group compared to the non-POP control group ($p < 0.0001$) [48,49]. Furthermore, data shows

that expression of ER β is lower and expression of miR-92 is higher in uterosacral ligaments of POP patients compared to non-POP controls. This suggests that ER β protein levels are lower secondary to increased miR-92 expression [50].

Contrary to the studies described above, other studies have shown opposite findings. Dietrich et al. found that mRNA levels of both ER α ($p < 0.001$) and ER β ($p = 0.057$) are higher in uterosacral ligaments from women with POP compared to non-POP controls [51]. Moreover, Zbucka-Kretowska et al. divided women into four groups to further study ER expression in vaginal walls: premenopausal women with POP, premenopausal women without POP, postmenopausal women with POP, and postmenopausal women without POP. Higher ER α and lower ER β expression are found in premenopausal women with POP compared to premenopausal women without POP. However, ER α and ER β expression did not differ between postmenopausal women with and without POP [52]. Another study further confirmed the majority of these findings, with the only difference being higher ER α expression in postmenopausal women with POP than in postmenopausal women without POP [45]. Elevated ER α expression has also been found in the cervix and levator ani. The levels of ER α in the cervix of women with POP are elevated compared to the women without POP [53]. The expression of ER α in the levator ani muscle stroma is also significantly higher in women with POP compared with age-matched asymptomatic controls [54].

Why are there conflicting results regarding ER expression in the pelvic connective tissues of women with POP? Differences in sample sizes, techniques, tissue types, menopausal status, and degree of POP may affect ER expression (Table 1) [45-58].

| | Sample size | | Menopausal status | Stage | Techniques | Tissues | Results | |
|---------------------------------|----------------------|-----------------------|-------------------|---------------|--------------------|--|-------------------------------|-------------------------------|
| | POP | Control | ER α | | | | ER β | |
| Grigoriadis C, et al. 2016 | 40 | 40 | Post | II and above | IHC | Pubocervical fascia | Low | Low |
| Shi Z, et al. 2012 | 40 | 40 | Post | II and above | Immunoblotting IHC | Uterosacral ligaments | Low | |
| Dietrich W, et al. 2012 | 13 | 13 | Pre & Post | II and III | RT-PCR | Uterosacral ligaments | High | High |
| Zbucka-Kretowska M, et al. 2011 | 4 (Pre) 12 (Post) | 5 (Pre) 9 (Post) | Pre & Post | uncertain | RT-PCR | Vaginal walls | High | Low (Pre) No change (Post) |
| Ibeanu OA, et al. 2010 | 14 | 28 | uncertain | uncertain | IHC | Cervix | High | |
| Bai SW, et al. 2005 | 20 | 24 | Post | III and IV | Immunoblotting | Uterosacral ligaments | Low | |
| Ewies AA, et al. 2004 | 8 (Pre) 25 (Post) | 15 (Pre) 10 (Post) | Pre & Post | III | IHC | Cardinal ligaments | High | Low (Pre) No change (Post) |
| Lang JH, et al. 2003 | 7 (Pre) 18 (Post) | 12 (Pre) 20 (Post) | Pre & Post | I, II and III | IHC | Cardinal ligaments and uterosacral ligaments | Low (Pre) No change (Post) | |

| | | | | | | | | |
|---|----|----|------------|--------------|--------------------|---|------|-----|
| Copas P, et al. 2001 | 45 | 10 | Pre & Post | uncertain | IHC | Levator ani muscle and the overlying fascia | High | |
| He K, et al. 2016 | 49 | 25 | Pre & Post | II and above | Immunoblotting IHC | Uterosacral ligaments | | Low |
| Pre: pre-menopause; Post: post-menopause. | | | | | | | | |

Table 1: Summary of the recent studies for evaluating ER expression on POP.

Sample size: Two major problems are present in the aforementioned studies: small sample size and unbalanced groups. For instance, three studies above contain less than 10 samples either in POP group or in control group [45,49,52]. Small sample sizes suggest that the study findings may not be representative of and generalizable to the overall patient population. Additionally, unbalanced subject groups limit authors' ability to accurately compare subjects and controls. As an example, one study compared 45 subjects with POP to only 10 control subjects [54].

Techniques: Immunohistochemistry (IHC), immunoblotting, and/or reverse transcription-polymerase chain reaction (RT-PCR) are three major techniques used in these studies. Two studies employed RT-PCR to qualitatively detect the expression of ER mRNA [51,52], while the other studies used IHC and/or immunoblotting to detect the distribution and localization of ER proteins [45-50,53,54]. Differences in mRNA and protein levels between these techniques do not always parallel each other. As a result, utilizing different techniques makes comparison of results between studies difficult.

Tissues: Diverse tissue types such as those discussed below, have distinct ER distribution patterns and functions. Similarly, the distribution pattern and function of ERs in pelvic floor connective tissues are different.

- Vaginal wall:** ER α localizes to vaginal epithelial, stromal, and smooth muscle cells, whereas ER β is expressed in vaginal epithelial and vascular smooth muscle cells. ER α mediates estrogen function in the vaginal epithelium such as epithelial proliferation, stratification, and cornification [55]. It has been reported that estrogen plays a role in vaginal vascular tissue through both genomic- and non-genomic-mediated mechanisms. ER β , which is located in the vascular tissue, may mediate the genomic effects of estrogen on human vaginal blood vessels [56]. Fuermetz et al. demonstrated that ER α expression is increased and that ER β expression does not change after local estrogen administration [57].
- Uterosacral ligament:** Regardless of POP status, Dietrich et al. found that ER α is expressed in smooth muscle cells but not in endothelial cells of female uterosacral ligaments, whereas ER β is located in endothelial cells but not in connective tissue [51].
- Pelvic muscle:** ER can be detected in the nuclei of connective tissue cells and striated muscle cells of the levator ani muscle [58].

In conclusion, the expression of ERs in the various tissue types affected by POP is diverse, leading to distinct responses to both locally produced estrogen and circulating estrogen levels.

Menopause status: After menopause, women experience a decrease in estrogen levels. Among these studies, only three studies compared the menopausal status between the study group and control group

[45,49,52]. In addition, ER expression is closely related to a subject's number of postmenopausal years [49]. The number of postmenopausal years should also be taken into account in future studies.

Degree of POP: Subjects in five studies were women with stage II or greater POP. The POP stage of study subjects in three studies is unknown [52-54]. In addition, the POP group of one study contained patients from every stage of POP [49]. We postulate that choosing advanced stages of POP may be more suitable for ER measurement because subtle differences in ER expression may not be detected by current technology in the early stages of the disease. It is important to note that POP stage classification in these studies was not standardized. It is plausible that ER expression may vary with different stages of POP, which could explain, in part, the controversy of ER expression in POP tissue.

Progesterone receptor and androgens

Progesterone receptor (PR): Expression of PR, which has been defined as an estrogen-regulated gene, may also be different in tissues from women with POP. Multiple studies have shown distinctive changes in PR expression levels that parallel changes in ER levels in POP patients [45,46,48,57].

Androgens: Androgens (testosterone and androstenedione) are the precursor for estrogen biosynthesis. It has been established that pelvic floor muscles are sensitive to testosterone [59]. Therefore, androgens may play a role in the development of POP. Currently, there are only a few studies exploring the relationship between androgen, androgen receptor (AR), and POP development. One study showed that serum testosterone concentrations in subjects with POP are the same compared to control groups [60]. Moreover, no papers have been archived in PubMed describing the role of locally produced androgens in the development of POP. However, AR expression in the vaginal wall and cardinal ligament of POP patients has been shown to be significantly higher than in controls [45,60]. Hormone replacement therapy (estradiol and cyclic norethisterone) can decrease AR expression [45]. Due to limited information, further studies are needed to define the potential role of androgens in POP development.

Conclusion

Estrogen has a profound influence on pelvic collagen metabolism, including synthesis and degradation. Estrogen also enhances the replacement of aging collagen by down-regulating MMPs and increases cystatin C in fibroblasts and smooth muscle cells of the vagina, leading to an overall increase in the concentration of collagen cross-links. Furthermore, estrogen affects the cytoskeleton and cell morphology of fibroblasts. The role of estrogen in pelvic floor connective tissues is unquestionable, but the role of its receptors is perplexing. At this time, estrogen receptor function remains unclear,

and there is no conclusive evidence suggesting a causal role of estrogen in POP. In order to address this question, a well-designed clinical study should include a sufficient sample size, uniform POP classification standards, a comprehensive method to choose experimental groups, suitable techniques, and a wider selection of diverse tissue types to ensure more accurate results.

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