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REVIEW ARTICLE

Histology of the vaginal wall in women with pelvic organ prolapse: a literature review

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Abstract
Introduction and hypothesis The pathophysiology of pelvic organ prolapse (POP) is incompletely understood. The purpose of this study is to describe the current knowledge about histology of the vaginal wall and its possible involvement in the pathogenesis of pelvic organ prolapse.
Methods Eligible studies were selected through a MEDLINE search covering January 1986 to December 2012. The research was limited to English-language publications.
Results Investigations of changes in the vaginal tissue that occur in women with genital prolapse are currently still limited and produced contrary results. The heterogeneity of the patients and the control groups in terms of age, parity and hormonal status, of the localization of biopsies and the histological methods as well as the lack of validation of the quantification procedures do not allow clear and definitive conclusions to be drawn.
Conclusions This review shows that current knowledge of the histological changes observed in women with POP are inconclusive and relatively limited. More studies are needed in this specific field to better understand the mechanisms that lead to POP.

Keywords Vagina · Pelvic organ prolapse · Collagen · Elastin · Connective tissue · Extracellular matrix

Abbreviations

POP	Pelvic organ prolapse
ECM	Extracellular matrix
MMP	Matrix metalloproteinase
LOX	Lysyloxidase
GSI	Genuine stress incontinence
FBN	Fibrillin
Latent	TGF-β binding protein
BMP	Bone morphogenic protein
EFW	Elastic fiber width
PGP 9.5	Protein gene product 9.5

Introduction

Pelvic organ prolapse (POP) is a common problem that can have a serious impact on quality of life in women. The lifetime risk of undergoing surgery for prolapse or incontinence is 11% [1]. Environmental factors such as multiparity, trauma, obesity, and aging are important risk factors in developing POP. However, genetic predisposition is widely recognized as a determinant contributing factor to the progression of POP. The significant frequency of POP in genetic diseases of connective tissues such as Ehlers–Danlos, Marfan syndrome, and cutis laxa, as well as in models of

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mice deleted for extracellular matrix (ECM) molecules, points to connective tissue disorder as the most likely etiological factor in POP. It is also supported by the high recurrence of POP after traditional surgical repair (nearly 30%), using weak native tissue [2]. Structural components forming the vaginal wall and its supportive connective tissue condition their biomechanical properties. Their alterations are most probably involved in the physiopathology of POP and need to be better specified.

Assessing changes in the composition of the vaginal ECM in POP is challenging. The fibrillar components, collagen and elastin, are highly insoluble. Measuring hydroxyproline for quantifying collagen content will give a total amount in the tissue without any distinction between the various types and any picture of tissue organization. Similarly, elastin can be measured indirectly through their specific cross-links, a technique that can lead to misleading results if the cross-linking process is altered in POP. The vaginal ECM also contains less abundant molecules, such as the elastic fibers-associated microfibrils, small and large proteoglycans and hyaluronic acid that play a major role in the architectural organization of mechanically competent fibrillar elements and in signaling to the cells. Investigating gene expression at the mRNA level has tentatively circumvented these difficulties. However, this approach provides a snapshot of the current metabolic activities of the cells that can be unspecifically altered by the weakened mechanical resistance of the tissue, and does not trace the long-term history of the disease. Histological and immunohistochemical analyses, although semi-quantitative, have been widely used to study tissue alteration in POP.

The purpose of this study is to summarize the anatomy and current knowledge about histological alterations of the vaginal wall and their involvement in the pathogenesis of pelvic organ prolapse.

Materials and methods

We performed a MEDLINE search using the terms "vagina, vaginal wall, histology, morphometry, morphometric, pelvic organ prolapse, collagen, elastin, vaginal smooth muscle, connective tissue, extracellular matrix and biochemical properties". Free text and Mesh terms were used. The research was limited to English-language publications from 1986 to 2012.

Results

Anatomy of the vaginal wall

The human vagina is a fibromuscular canal composed of four layers. As can be seen in Fig. 1 showing a full-thickness section of the vaginal wall, the epithelial layer is a superficial nonkeratinized, stratified, squamous epithelium. The subepithelial layer or lamina propria is a dense connective tissue layer mainly composed of fibrillar collagens and elastin populated by fibroblasts. The lamina propria is perforated by small arterioles and venules. The muscularis is composed of inner circular and outer longitudinal smooth muscle cells surrounded by connective tissue. The adventitia is a loose connective tissue layer that separates the muscularis of the vagina and the paravaginal tissue. The lamina propria and muscularis are the two important layers that confer a great tensile strength to the vaginal wall [3]. Several authors investigated histology of the "endopelvic fascia" used by gynecological surgeons for colporrhaphy [4–6]. This fascia may not exist as a specific tissue, but represents the fibromuscular layer of the vaginal wall (muscularis and adventitia). The adventitia is variably discrete and does not form a true fascial layer.

Biochemical aspects of the vaginal connective tissue

The connective tissue of the vagina is composed in varying proportions of cellular elements, fibroblasts, and smooth muscle cells, surrounded by an extracellular matrix (ECM). Although fibroblasts are the main cells responsible for the synthesis and secretion of fibrillar (collagen and elastin) and less abundant non-fibrillar components, smooth muscle cells can also synthesize these molecules. Collagen and elastin are fundamental components that control the biomechanical properties of the vaginal tissue. Collagen fibers are very rigid and do not easily distort while elastic fibers provide elasticity and recoil to the tissue. The ECM is in constant remodeling, and its homeostasis depends on the balance between synthesis and degradation by matrix metalloproteinases (MMPs) further controlled by activators and inhibitors (TIMPs). Both processes are modulated not only by soluble biological mediators such as growth factors and their receptors, but also by the chemical and mechanical signaling issued from the ECM itself and recognized by transmembrane receptors to the ECM components, integrins. This latter mechanism participates in the degradation of the vaginal wall.

Collagen

Fibrillar collagens are the principal determinants of vaginal tissue strength. Collagen molecules are homo- or heterotrimers made of three polypeptidic α-chains coiled together to form a triple-helix [7]. These molecules spontaneously polymerize upon cleavage of the amino- and carboxy-terminal propeptides by specialized enzyme to form microfibrils, fibrils and bundles of fibrils progressively stabilized by cross-link upon the activity of lysyloxidase (LOX; Fig. 2) [8]. Collagen I, III, and V are the main collagen subtypes

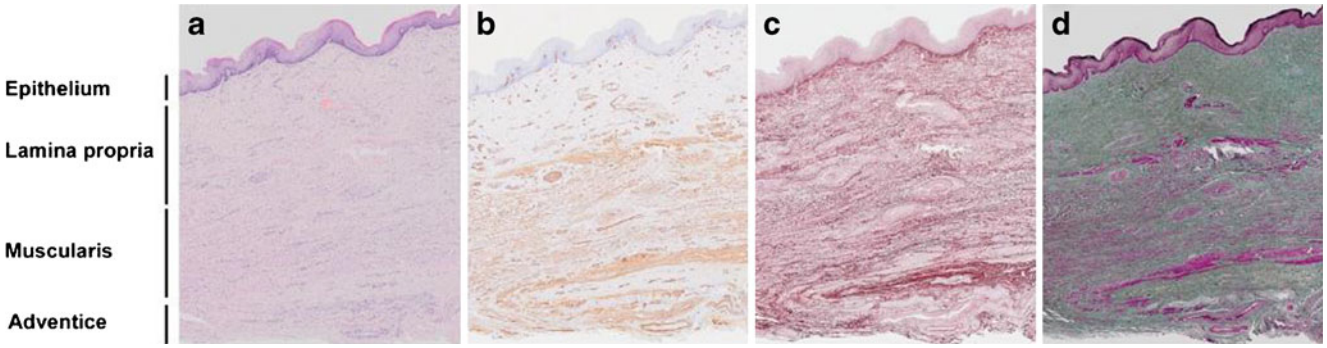

Fig. 1 Histology of full-thickness biopsy specimens obtained from cross-sections of the anterior vaginal wall in women with pelvic organ prolapse. The vaginal wall consists of four layers: the epithelium and lamina propria (vaginal mucosa), muscularis, and adventia. **a** Hematoxylin–eosin staining, **b** staining for smooth muscle α-actin, **c** orcein stain for detection of elastic fibers, **d** Masson's trichrome stain. Original magnification, ×10

present in the vagina. Collagen I forms large and strong fibers responsible for the mechanical resistance of the tissue. This subtype is abundant in skin, ligament, tendon, and bone. Collagen III forms smaller fibers of lower tensile strength and is present in mobile organs and cyclically stretched tissues such as blood vessels [9]. Type I collagen confers strength to tissues while type III contributes to elasticity. Type V collagen forms small fibers of low tensile strength. Collagens I, III, and V copolymerize to form hybrid fibrils. Type V collagen forms the core of the fibril surrounded by co-polymers of collagen type I and type III. The proportion of each subtype of collagen determines the fiber size and has an impact on the biomechanical strength of the tissue [10, 11]. The collagen fibers are further covered by FACIT collagens XII and XIV and small leucine-rich proteoglycans such as decorin, which also participate in the control of fibrillogenesis.

Several studies have analyzed changes in the vaginal connective tissue of patients with and without POP by histology [12–19] using various methods and stainings to assess changes in collagen content in the vaginal wall. The results of these studies are described in Table 1.

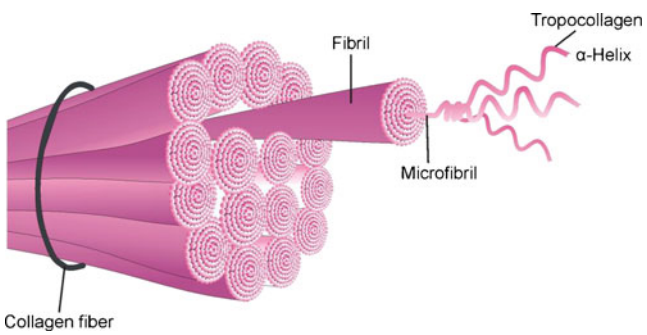

Fig. 2 The structure of collagen. Triple helical collagen molecules (tropocollagen) polymerize to form microfibrils, fibrils, and bundles of fibrils (collagen fiber) progressively stabilized by cross-link upon the activity of lysyloxidase (LOX)

Makinen et al. reported an increased amount of collagen fibers and a rarefaction of fibroblasts in the vaginal fascia of women with POP, using a Weigert Van Gieson stain [12]. In agreement with this study, Kökçü et al. compared connective tissue components of the vaginal tissue samples in the precervical region with Gomori's one-step trichrome and described higher "scores" of collagen and lower "scores" of fibroblasts in patients with POP [13]. They postulated, although with no supportive data, that the fibroblasts in patients with POP might be producing a high amount of weak collagen, probably type III, leading to pelvic support disorders.

Picrosirius red stain can also be used to visualize collagen fibers from the vaginal tissue. Contrasting with the above studies, Takano et al. did not find any significant difference between the POP and control groups [14]. By using a Picrosirius polarization method, Borges et al. observed a molecular disorganization and fragmentation of the collagen fibers in the lamina propria of women with POP [15].

Liapis et al. analyzed type III collagen in the vagina around the urethra by immunohistochemistry and reported a severely reduced visual score in patients with POP and genuine stress incontinence (GSI) and a moderate reduction in patients with POP alone compared with the control group [16]. From the study by Goepel et al. who investigated several collagen types in the periurethral vagina wall in continent and incontinent postmenopausal women with POP, it seems that tissues from POP patients with GSI showed weaker immunostaining for types I, III, and IV collagens [17]. In agreement with these results, by investigating the same molecules and analyzing the immunostaining by automatic digital image analysis, Lin et al. observed a significant reduction in only collagen III in the apex of the anterior vaginal wall in patients with POP [18].

In contrast to these results, Moalli et al., by using semi-quantitative immunofluorescence staining for collagen types I, III, and V in full-thickness biopsies of the vaginal apex,

Table 1 Collagen analysis by histology in biopsy specimens from vaginal tissue in patients with prolapse or without prolapse

Study	Target population and sample size	Menopausal status	Mean age (years)	Parity	Biopsy localization	Histological localization	Analytical methods	Findings: patients with POP compared with controls
[12]	10 women with POP	Not described	60.1	Not described	Anterior precervical vaginal fascia	Not described	Histology with HE, Weigert Van Gieson, PAS and Gomori	Increased collagen fibers and decreased amount of fibroblasts
	10 controls		47.3					
[16]	34 women with SUI and POP	16 pre- and 18 post-menopausal	54.4±7.1	2.4±1.2	Anterior paravaginal fascia at the level of the bladder neck	Not described	Immunohistochemistry	Decreased collagen III in women with SUI and prolapse, no significant difference in women with prolapse alone compared with controls
	32 women with POP	15 pre- and 17 post-menopausal	55.4±7.4	2.5±1.1				
	28 controls	10 pre- and 18 post-menopausal	53.6±6.7	2.3±1.0				
[13]	24 women with POP	Premenopausal	44.4±5.2	3.0±1.1	Precervical vaginal fascia	Not described	Histology with HE, Gomori's trichrome	Increased total collagen (subtypes not assessed)
	21 controls	Premenopausal	45.6±4	2.9±0.8				
[14]	45 women with POP	22 premenopausal	42.7 (35–49)	Not described	Fragments of vaginal apex	Not described	Histology with Picrosirius	No statistically significant difference in total collagen between the groups
		23 postmenopausal	65.6 (47–85)					
	10 controls	Premenopausal	41.9 (32–50)					
[17]	29 patients with POP (15 with SUI and 14 without SUI)	Postmenopausal	61.9 (49–74)	Not described	Periurethral vaginal fascia	Mucosa	Immunohistochemistry	Decreased collagen I, III, and VI in women with SUI. No difference in collagen IV and V
[19]	37 patients with POP	11 premenopausal	42.7±7.1	2 (1.4)	Full-thickness vaginal apex at one of the lateral fornices	Subepithelium and muscularis	Histology, laser scanning confocal microscopy and immunofluorescence	Increased collagen III with no difference in collagens I and V
		13 postmenopausal (no HT)	68.1±9.4	3 (2.4)				
		13 postmenopausal (on HT)	64.4±8.6	3 (2.4)				
	11 controls	Premenopausal	39.7±7.1	3 (1.4)				
[15]	6 women with POP	Postmenopausal	Not described	Not described	Fragments of vaginal apex	Mucosa	Picrosirius polarization staining method	Collagen fiber disorganization
	6 controls	Not described	Not described					
[18]	23 women with POP	8 pre- and 15 post-menopausal	57.4±15.1	4 (2–12)	Full-thickness precervical anterior vaginal wall	Not described	Immunohistochemistry	Decreased collagen III, collagen I and III had significant positive correlations with ageing
	15 controls	13 pre- and 2 post-menopausal	47.1±15.5	3 (0–7)				
[31]	20 women with POP	Not described	61	1–4	Full-thickness "redundant" and non-prolapsed vaginal tissue	All Layers	Histology with HE, Masson's trichrome, Verhoeff Van Gieson, and immunohistochemistry	No significant differences in collagen content between prolapsed and nonprolapsed tissues

POP pelvic organ prolapse, *SUI* stress urinary incontinence, *HE* hematoxylin and eosin, *PAS* periodic acid-Schiff

observed that type III collagen is the major fibrillar collagen of the vagina in patients with POP, independent of age and parity [19]. Furthermore, the total amount of collagen was increased in women with POP relative to women without POP, primarily because of increased expression of type III collagen. They postulated that the predominance of type III collagen might explain the increased flexibility and distensibility and the decreased tensile strength and therefore contribute to the progression of the disease.

These conflicting results further support the need for well-controlled studies in terms of patients, anatomical and histological definition of the tissue, and validated quantification methods.

Elastin

Elastic fibers are key architectural elements of connective tissues that are subject to mechanical strain and expansile forces. They provide extensibility and recoil to elastic tissues. This property of resilience is important for the maintenance of vaginal structural integrity against mechanical strain.

Elastin fibers are formed through a highly regulated and stepwise process including the initial formation of a microfibrillar scaffold, the guidance and deposition of tropoelastin monomers into the scaffold followed by their enzymatic cross-linking to form the functional insoluble elastin polymer (Fig. 3).

More than 30 elastic fiber-associated proteins have been identified, among which the major microfibrillar components fibrillin 1 and 2 (FBN), the cross-linking lysyl oxidases 1–4 (LOX), the lysyl oxidase-like 1 (LOXL1), and proteins of the fibulin 1–5 family. Further to their structural role, the fibrillin-rich microfibrils possess signaling functions through their ability to bind latent TGF-β-binding proteins (LTBPs) and bone morphogenic proteins (BMPs) and regulate their bioavailability.

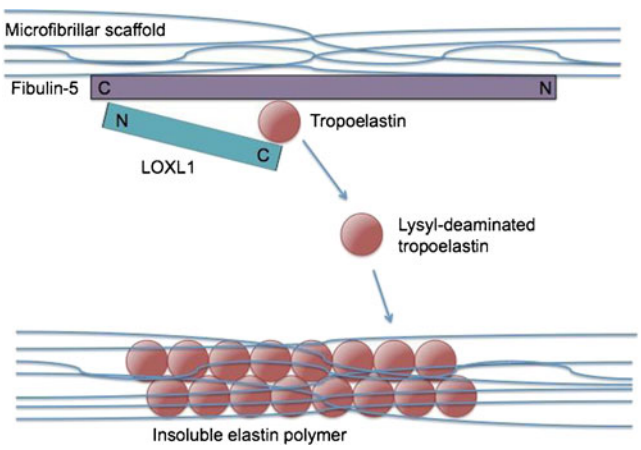

Fig. 3 A simplified model of elastogenesis. Elastin fibers are formed through a highly regulated and stepwise process including the initial formation of a microfibrillar scaffold, the deposition of tropoelastin monomers cross-linked together in a reaction catalyzed by lysyl oxidase

Several human connective tissue diseases linked to genetic alterations of molecules forming elastic fibers such as Marfan syndrome (FBN1) or cutis laxa have been associated with a high incidence of POP. This finding has motivated gene targeting studies of the proteins involved in elastogenesis [20, 21]. Elastogenesis starts at midgestation and completes during postnatal development. It is generally admitted that no new elastic fiber is formed in the adult with the exception of a remodeling of the pelvic organs during pregnancy and postpartum recovery. LOXL1 and fibulin-5 seem to be most significant factors in vaginal remodeling after parturition and in age-associated POP. A large proportion of knock-out *Loxl1* $^{-/-}$ mice develop spontaneous POP at the age of 12 weeks or after the first and second delivery and have a significantly lower vaginal elastin content than the wild-type animals. An even higher proportion of fibulin-5 knock-out mice (90%) develop prolapse with advancing age in relation to an abnormal elastic fiber formation associated with increased degradation due to MMP9 elastolytic activity up-regulation by loss of the negative feedback control operated by fibulin-5 on MMP9. These mutant models give insights into the mechanisms controlling homeostasis of the elastic fibers and potentially underlying pelvic prolapse in women

Measuring the elastin at the mRNA level in the vaginal wall probably does not reflect the actual status of elastin in the tissue as several post-transcriptional and translational mechanisms can regulate its turnover. A few studies have investigated the elastin changes in women with POP by immunohistochemistry (Table 2) [18, 22, 23].

According to Karam et al., who compared elastin expression and elastic fiber width (EFW) in the anterior vaginal wall of postmenopausal women with or without anterior prolapse [22], patients with POP had lower elastin expression and lower EFW than controls. They also found that EFW did not correlate with age in these postmenopausal women. On the contrary, Lin et al. found by immunohistochemistry high elastin expression in the POP group, but after correcting for age and menopausal status there was no difference between the two groups [18].

Fibulin-5 expression has also been investigated by immunohistochemistry in women with or without anterior vaginal wall prolapse [23]. Staining intensity for fibulin-5 was diminished in the POP group compared with controls.

The better understanding of the critical role played by elastin-associated proteins not only in the formation of elastic fibers and their ability to regulate the activity of growth factors, but also in the control of their degradation by MMPs, should open new methods of investigation in the physiopathology of POP.

Table 2 Elastin analysis by histology in biopsy specimens from vaginal tissue in women with or without prolapse

Study	Target population and sample size	Menopausal status	Mean age (years)	Parity	Biopsy localisation	Histological localization	Analytical methods	Findings: patients with POP compared with controls
[13]]	24 women with POP 21 controls	Premenopausal Premenopausal	44.4±5.2 45.6±4	3.0±1.1 2.9±0.8	Precervical vaginal fascia	Not described	Histology with Verhoeff Van Gieson elastic stains	No statistically significant difference in elastin content
[18]	23 women with POP	8 pre- and 15 post-menopausal	57.4±15.1	4 (2–12)	Full-thickness precervical anterior vaginal wall	Not described	Immunohistochemistry	No statistically significant difference in elastin content
	15 controls	13 pre- and 2 post-menopausal	47.1±15.5	3 (0–7)				
[22]	33 women with POP 10 controls	Postmenopausal Postmenopausal	70.5 (62–78) 70.5 (56–76)	2.5 (2–4) 3 (1.2–3)	Full-thickness upper lateral anterior vaginal wall	Muscularis	Immunohistochemistry	Decreased elastin content and fiber width
[28]	12 women with POP	8 pre- and 4 post-menopausal	54±7	2 (1–6)	Full-thickness precervical anterior vaginal wall	Not described	Immunohistochemistry	Decrease staining intensity for fibulin-5
	10 controls	5 pre- and 5 post-menopausal	49±4	2 (0–5)				
[31]	20 women with POP in prolapsed and nonprolapsed localization	Not described	61	1–4	Full-thickness "redundant" and nonprolapsed vaginal tissue	All layers	Histology with Verhoeff Van Gieson elastic stains	No significant differences in elastin content between prolapsed and nonprolapsed tissues

Morphometric analysis of the vaginal wall

Little is known about the changes in the histomorphometry of the vaginal wall in women with POP. Hypoestrogenism is an important cause of structural modifications in the vaginal wall [24]. Urogenital atrophy and reduction in vaginal wall thickness has been reported to occur after menopause, although without precise morphometry. To date, the histological and morphometric analysis of the vaginal wall in women with POP is still inconclusive.

Histological analyses of the vaginal tissue of patients with or without POP can be used to observe changes in the different components (collagen, elastin, smooth muscle cells, vessels or innervation) of the tissue. Tulikangas et al. used histology to compare the vaginal wall of women with or without enterocele and observed an increase in vaginal wall muscularis thickness in the POP group [25].

Boreham et al. were the first to specifically evaluate the histomorphometric features of the anterior and posterior vaginal wall in women with and without POP [6, 26]. They observed that the morphometry of the vaginal wall muscularis is significantly altered in women presenting POP, with a decrease in the fractional area of smooth muscle in the muscularis layer and disorganized smooth muscle bundles. They suggest that the decreased staining of α-actin in prolapsed vagina may represent a loss of the differentiated phenotype of the smooth muscle cells or may result from apoptosis of smooth muscle cells. They did not find any difference in apical vaginal tissue thickness between the two groups.

Many other reports have published concordant results with the study by Boreham et al. (Table 3) [27–30]. The fraction of smooth muscle in the muscularis of the anterior vaginal wall was significantly decreased in women with POP and there was no significant difference between primary and recurrent cases. Takacs et al also observed a statistically significantly increased smooth muscle cell apoptotic index in women with POP, suggesting that a decrease in the amount of smooth muscle in the anterior vaginal wall might be due to accelerated apoptosis [28].

Another original approach is to compare the histology of the vaginal wall in two different locations from the same patient to determine whether histological differences were due to intra- or inter-individual variability. Kannan et al. found that histological differences are very subtle and that both prolapsed and nonprolapsed tissues expressed the same changes in collagen, elastin or smooth muscle content [31].

Measurement of the distance between the surface epithelium and the nearest point of the muscularis have also been used to analyze changes in the vaginal tissue of women with or without POP [29, 30]. The mean distance of the smooth muscle fibers from the surface epithelium of the prolapsed group was significantly higher than in the control group. Da

Table 3 Smooth muscle analysis by histology in biopsy specimens from vaginal tissue in women with or without prolapse

Study	Target population and sample size	Menopausal status	Mean age (years)	Parity	Biopsy localisation	Histological localisation	Analytical methods	Findings: patients with POP compared with controls
[25]	13 women with POP	2 pre- and 11 post-menopausal (8 on HRT and 3 no HRT)	66±11	3.7±1.2	Full-thickness vagina at the leading edge of the enterocele	All layers	Histology with Movat's pentachrome	Increased muscularis thickness, no statistically significant difference in the vaginal wall thickness among the three groups
	5 women undergoing hysterectomy	Not described	Not described					
	13 women undergoing radical hysterectomy	Not described	Not described					
[6]	28 women with POP	14 pre- and 14 post-menopausal (5 on HRT and 9 no HRT)	49.3±2.6	3.6±0.3	Full-thickness anterior vaginal apex	Muscularis	Immunohistochemistry (A-SMA)	Decreased fraction of nonvascular smooth muscle
	12 controls	Premenopausal	39.5±1.5	2.4±0.4				
[26]	15 women with POP	11 pre- and 4 post-menopausal (4 on HRT)	45.6±3.0	3 (0–6)	Full-thickness posterior vaginal apex	Muscularis	Immunohistochemistry (A-SMA)	Decreased fraction of nonvascular smooth muscle
	8 controls	Premenopausal	38.5±2.8	2 (0–3)				
[27]	11 women with POP	Postmenopausal (3 on HRT)	68.4±7.8	2.5±1.1	Full-thickness anterior vaginal apex	Muscularis	Immunohistochemistry (A-SMA)	Decreased fraction of nonvascular smooth muscle
	8 controls	9 pre- and 2 post-menopausal (1 on HRT)	45.3±8.0	2.0±1.2				
[28]	6 women with POP	Postmenopausal	61±3	1 (1–4)	Full-thickness anterior vaginal apex	Muscularis	Immunohistochemistry (A-SMA)	Decreased fraction of nonvascular smooth muscle
	6 controls	1 pre- and 4 post-menopausal	46±5	1 (0–5)				
[32]	31 women with POP	18 Premenopausal	35.67±4.29	4.7±1.54	Full-thickness anterior and posterior vaginal wall	All layers	Histology with Masson's trichrome	Increased muscularis thickness and total vaginal thickness in the postmenopausal group
		13 Postmenopausal	53.64±5.36	2.8±0.81				
[29]	49 women with POP	20 pre- and 29 post-menopausal (9 on HRT)	53.94±10.4	3 (1–9)	Full-thickness anterior middle portion (Aa) of the vagina	All layers	Immunohistochemistry (A-SMA)	Decreased smooth muscle content in the muscularis, increased thickening of the subepithelium
	40 controls	32 pre- and 8 post-menopausal (7 on HRT)	46.5±4.03	2 (1–4)				
[30]	37 women with POP	11 pre- and 26 post-menopausal	59±1.09	4 (1–9)	Full-thickness anterior vaginal apex	All layers	Immunohistochemistry (A-SMA)	No statistically significant difference in smooth muscle content, increased thickening of the subepithelium
	47 controls	41 pre- and 6 post-menopausal	46.40±5.92	2 (0–5)				
[31]	20 women with POP in prolapsed and nonprolapsed localization	Not described	61	1–4	Full-thickness "redundant" and nonprolapsed vaginal tissue	All Layers	Immunohistochemistry (A-SMA)	No significant differences in smooth muscle content

Silva et al measured vaginal wall thickness in pre- and postmenopausal women who underwent surgery for genital prolapse with grades I–II [32]. The vaginal wall was thicker in the postmenopausal than in the premenopausal group. These thicknesses seem to be due to the muscularis layer, which was also thicker in the postmenopausal group, possibly because of the accumulation of fibrosis in this layer. However, a precise measurement of total vaginal wall thickness is difficult to obtain from vaginal samples because cross-sections must be perfectly perpendicular to the tissue edge. Oblique sections would appear thicker than those perpendiculars.

Vascularization of the vaginal wall

Vascularization of the vaginal wall is extensive. Arteries arise between the muscularis and adventitial layers and spread in a richly developed subepithelial capillary network forming a venous plexus of small veins that drain into larger veins crossing into the muscularis to reach the adventitia.

Few studies have examined the potential difference in vascularization of the vagina between women with and without POP. Boreham et al. observed more frequently large dilated venules in the lamina propria of women with advanced stage anterior prolapse, suggesting that this abnormal vaginal vascularity may result from increased stasis and gravity on the prolapsed tissue [6]. The poor muscularis contractility may contribute to altered mechanical modulation of venous outflow in the prolapsed organ. The fractional area of the blood vessel was similar in the report by Badiou et al. [27].

Lin et al. observed the capillaries surrounding the arterioles in the vaginal wall and found that the number of capillaries surrounding arterioles in women with anterior vaginal prolapse was significantly less than that of the control group ($p<0.05$) [18]. They suggested that anterior vaginal wall prolapse might result in attenuating blood supply in the upper vagina.

Innervation of the vaginal wall

The innervation of the vaginal wall in patients with POP is poorly evaluated. The integrity of the vagina and its supportive connective tissue is essential for a normal pelvic floor function and anatomy of the pelvic organs. Branches of the hypogastric plexus innervate both the levator ani and the posterior vagina [33]. Denervation injury of the pelvic floor during labor may contribute to loss of vaginal support and therefore may lead to POP [34, 35].

Several studies have evaluated the innervation of the anterior vaginal wall in women with or without POP. Zhu et al. have analyzed the staining of protein gene product 9.5 (PGP 9.5), a neuronal marker for peripheral nerves and ganglia in tissues [36]. They showed that the nerve fibers profile of vaginal epithelium and subepithelium in women with SUI and POP were significantly lower than those in the control group. By evaluating the anterior vaginal wall innervation using PGP 9.5, Inal et al. observed a decreased number and diameter of the subepithelial nerve fibers in the anterior vaginal wall of women with anterior prolapse than in women with normal vaginal support [29]. Kaplan et al. recently confirmed their findings by describing decreased neuronization in the vaginal wall in the POP group [30].

To date, only two studies have evaluated the innervation of the posterior vaginal wall in women with or without POP. Boreham et al. have used antibodies to S100, a marker that recognizes glial cells and astrocytes, and found that nerve bundles were smaller and fewer in the vaginal muscularis of women of the POP group [26]. Altman et al. found opposite results by using PGP 9.5 antibodies, with an increased nerve fiber density in the subepithelium of the rectovaginal wall of patients with posterior vaginal wall prolapse. They postulated that a compensatory neural regeneration following nerve trauma might play a role in the pathogenic process of pelvic floor disorders [37]. These conflicting results may also be explained by the difference in the method of tissue sample collection, localization and the sensitivity of these two neuronal markers.

Discussion

The pathophysiology of pelvic organ prolapse is multifactorial. Multiparity, trauma, obesity, and aging are associated with connective, muscular, and neuromuscular alterations of the pelvic tissues that are linked to the development of POP. Besides the role of these environmental factors, intrinsic connective tissue disorder is an important contributing factor to the pathogenesis of POP.

Investigations of changes in the vaginal tissue that occur in women with genital prolapse are currently still limited. Several studies have analyzed collagen content or changes in collagen subtypes in the vagina of patients with prolapse compared with controls and produced contrary results. These conflicting results can be explained by the different methods of collagen quantification used in these reports.

Studies on the histomorphometry of the vaginal tissue also found inconclusive results. The biopsy site may vary from one study to another and specimens are not often well defined by histology, making it impossible to determine exactly which layer of the vaginal wall is being analyzed. It is therefore difficult to make comparisons between current studies. Moreover, in some studies, the histological sections are interpreted subjectively by pathologists or measured on randomly selected fields, which can lead to bias. Indeed, the proportions of each constituent of the tissue may vary depending on the layer studied and from one place to another within the same layer.

In most of these studies, the population present large variations in age and menopausal status. Patients in the POP group are older in the postmenopausal period than those in the control group. It is therefore difficult to determine whether these changes are related to POP or rather to ageing. In addition, studies are often conducted on a relatively small number of patients making it difficult to draw conclusions.

Several studies suggest that changes in the morphometry of the vagina, particularly at the muscularis layer, may affect the function of the tissue and contribute to the development of POP [6, 28]. Although these changes are often observed, the mechanism of this transformation is still unclear. On the other hand, some authors have also found normal vaginal tissue in patients with POP, suggesting that the pathophysiology of POP cannot be explained only by an alteration of the quality of the vagina [12].

According to other reviews, the heterogeneity of the patients and the control groups in terms of age, parity, and hormonal status, of the localization of biopsies and the histological methods, as well as the lack of validation of the quantification procedures do not allow clear and definitive conclusions to be drawn [38, 39]. With the present state of knowledge, it is unclear whether the changes observed in the vaginal tissue are a cause or an effect of POP. Therefore, it will be useful to integrate these concepts into studies using molecular biology and experimental basic research. Another line of research is to correlate these results with the biomechanical properties of the tissue to help determine the role of each component in the changes that we observe in POP.

Conclusion

This review shows that current knowledge of the histological changes observed in women with POP are inconclusive and relatively limited, probably because of the lack of standardization of biopsy sites and methods of quantification. More studies are needed in this specific field to better understand the mechanisms that lead to POP.

Conflicts of interest The authors have no conflicts of interests, except Michel Cosson, who is a consultant for Ethicon, AMS, Boston, Ipsen, Olympus and is a researcher for Ethicon.

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