Histology of the vaginal wall in women with pelvic organ prolapse

Dr Laurent de Landsheere
Belgian Menopause Society, may, 2018
Histology of the vaginal wall

- Introduction

- Histology of the vaginal wall in women with POP
  - Histological properties
  - Bioméchanical properties

- Histology of the vaginal wall in post-menopause

- Laser rejuvenation
  - New trend or real benefit?
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Histology of the vaginal wall

Introduction

Muscularis
- Smooth muscle cells
- Connective tissue

Adventice
- Loose connective tissue

Lamina propria
- Dense connective tissue
  - Collagen
  - Elastin

Non kerat squamous epithelium
Introduction

Histology of the vaginal wall

- **Specific stainings**
  - Hematoxylin eosin, Orcein and Miller for Elastin, Masson’s Trichrome

- **Immuno-histochemistry**
  - Smooth muscle cells, Collagen fibers

### Diagram:

- **Epithelium**
- **Lamina propria**
- **Muscularis**
- **Adventice**

**Images:**

- A: Hématoxyline Eosine
- B: α-Smooth Actin (α-SMA)
- C: Orcéine
- D: Trichrome de Masson
Collagen

- Collagen I: large and strong fibers, mechanical resistance
- Collagen III: smaller fibers, low tensile strength (mobile organs)
- Collagen V: small fibers of lower tensile strength

Collagen I, III and V copolymerize to form hybrid fibrils.
Introduction

Elastin

- Key architectural elements of connective tissues that are subject to mechanical strain and expansile forces,
- Important for the maintenance of vaginal structural integrity
  - Low rigidity fibers
  - Large ability of extensibility and recoil, without rupture

Coloration à l’orçéine (grossissement X40)
Introduction

Collagen - Elastin

Figure 6: Illustration de faisceaux de collagènes et de fibres d'élastine par microscopie électronique à balayage. (Plérand et al, Microanatomy of the dermis in relation to relaxed skin tension lines and Langer's lines, Am J Dermopathol, 1987 Jun;9(3):219-24).
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Pelvic organ prolapse

Definition

- Descent of the pelvic organs from their normal position

Cystocele
Risk factors

Facteurs acquis
- Vieillissement, ménopause
- Obésité, constipation, tabac, sport
- Hystérectomie

Facteurs génétiques
- Marfan, Ehlers-Danlos, Cutis Laxa
- Altération métabolisme MEC (collagène, élastine)

Facteurs liés à la reproduction
- Multiparité, macrosomie
- Traumatismes obstétricaux (Forceps, …)
Anatomical aspects

- The maintain of pelvic floor, resulstance of 3 systems

**Level 1**
Suspension of vaginal apex
Cardinal and sacro-uterine ligaments

**Level 2**
Cohesion between vagina and levator ani aponeurosis
Endopelvic fascia, arcus tendineus

**Level 3**
Support of inferior third vagina
Perineal muscles, perineal body

The vagina and its connections play an important in the pelvi-perineal balance
Histological aspects

- What are the changes in the vaginal tissue in case of POP?
  - Contradictory results
    - ↓ or no ≠ in total collagen content
    - ↑ or ↓ collagen III density
    - ↓ or no ≠ in elastin density
  - High variability in the methodology
Pelvic organ prolapse

Study of the histological properties

- Tissue collection
  - Mid vagina and Apical vagina
Morphometric analysis of vaginal tissue

- Individualization of the vaginal layers

Immuno-marquage par α-SMA, grossissement X10 (LBTD)
Morphometric analysis of the vaginal tissue

- Measurement of smooth muscle cells density in the muscularis layer
Morphometric analysis of the vaginal tissue

- Measurement of vessels density in lamina propria and muscularis

Immuno-marquage par α-SMA, grossissement X10 (LBTD)

Densité de vaisseaux dans la lamina propria

Densité de vaisseaux dans la couche musculaire
Pelvic organ prolapse

Morphometric analysis of the vaginal tissue

- Measurement of elastin density in the lamina propria and muscularis

Coloration des fibres élastiques par l’orcéine, grossissement X10 (LBTD)

Densité d’élastine dans la lamina propria

Densité d’élastine dans la couche musculaire
Morphometric analysis of the vaginal tissue

- Comparison between Mid vagina and apical vagina

Portion moyenne du vagin

Région péricervicale
"Changes in elastin density in different locations of the vaginal wall in women with pelvic organ prolapse"

<table>
<thead>
<tr>
<th></th>
<th>Lamina propria</th>
<th>Muscularis layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Middle third area</td>
<td>Precervical area</td>
</tr>
<tr>
<td><strong>Mean collagen density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior vaginal wall</td>
<td>88.6±1.2</td>
<td>84.3±1.9</td>
</tr>
<tr>
<td>Posterior vaginal wall</td>
<td>87.8±1.5</td>
<td>90.9±0.6</td>
</tr>
<tr>
<td><strong>Mean elastin density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior vaginal wall</td>
<td>8.4±1.2</td>
<td>12.1±2.0</td>
</tr>
<tr>
<td>Posterior vaginal wall</td>
<td>6.8±0.5</td>
<td>10.1±1.4</td>
</tr>
<tr>
<td><strong>Mean blood vessels density</strong></td>
<td></td>
<td></td>
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<tr>
<td>Anterior vaginal wall</td>
<td>2.7±0.2</td>
<td>2.9±0.2</td>
</tr>
<tr>
<td>Posterior vaginal wall</td>
<td>2.1±0.3</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td><strong>Mean smooth muscle cells density</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior vaginal wall</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Posterior vaginal wall</td>
<td>—</td>
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</tr>
</tbody>
</table>

Data are presented in percent (mean ± SD)
*Statistically significant

Pelvic organ prolapse

“Changes in elastin density in different locations of the vaginal wall in women with pelvic organ prolapse”

Significant decrease of elastin density in the most dependent portion of the vaginal wall

Pelvic organ prolapse

Biomechanical properties of vaginal tissue

- Uniaxial traction testing until rupture

Intron 5882

Rubod et al, J Urol, 2007
Biomechanical properties of vaginal tissue

- Stress – strength curves
- Very high interindividual variability
- Non linear relationship between stress and strain

Pelvic organ prolapse

Rubod et al, J Urol, 2007
Pelvic organ prolapse

Biomechanical behaviour

Non linear behaviour

hyperelastic

Mooney-Rivlin Model

\[ \sigma = 2 \left( \lambda - 1 / \lambda^2 \right) [C_0 + C_1 \left( \lambda^2 + 2 / \lambda - 3 \right)] \]

- \( C_0 \) Rigidity at low deformation
- \( C_1 \) Rigidity at high deformation

Rubod et al, Int Urogynecol J, 2008
Pelvic organ prolapse

Elastin density: Link between histological and biomechanical properties of vaginal tissue in women with pelvic organ prolapse?

Laurent de Landsheere¹,², Mathias Briës³, Silvia Blacher³, Carine Munaut³, Betty Nuygen⁴, Chrystèle Reboul⁴, Agnès Noel⁴, Jean-Michel Foidart¹,², Michèle Nicole⁵, Michel Canon⁵

Propriétés biochimiques du tissu vaginal

Propriétés biochimiques du tissu vaginal

Significant inverse correlation between $C_0$ and the elastin/collagen ratio in the lamina propria.
The biomechanical properties of vaginal tissue are variable from one patient to another and the main predictor of the rigidity of the vagina is the density of elastin.
Pelvic organ prolapse

Conclusion

- POP is a common pathology and its pathophysiology is **multifactorial**

- A loss of elasticity of the vaginal tissue results, in biomechanics, by a greater rigidity tissue

- **Elastin** is a key element in maintaining the integrity of the vagina
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Anatomical and physiological changes

- Decrease estrogen levels and aging
  - Vulvo-vaginal atrophy (Genitourinary Syndrome)
    - Thinning of the top layer of superficial epithelial cells
    - Loss of elasticity of the vaginal epithelium
    - Increase subepithelial connective tissue (lamina propria)
    - Loss of rugae
    - Shortening and narrowing of the vaginal canal
    - Decrease in vaginal secretions
    - Increase in vaginal PH to $\geq 5$

Histology of the vagina in Menopause

JAMA, 2016
Clinical manifestation of genitourinary Syndrome

- Vaginal atrophy
  - Dryness
  - Burning
  - Pain, irritations, especially during sexual intercourse
  - Discharge, bleeding

- Urinary symptoms
  - Increase urinary frequency
  - Urge incontinence
  - Infection

Histology of the vagina in Menopause

JAMA, 2016
Lifetime changes of the vaginal tissue

- The Vaginal mucosa
  - (A) Pre-menopausal, (B) Moderate atrophy, (C) Severely atrophic

Tadir et al, Laser in surgery, 2017
Lifetime changes of the vaginal tissue (sheep)

- Epithelium and lamina propria
  - PAS = Periodic Acid Schiff: detection of glycogen (Dark superficial purpel layer)

The thickness of glycogen staining epithelial layers changed with puberty and menopause. The epithelium was markedly thicker after multiple deliveries.
Lifetime changes of the vaginal tissue (sheep)

- Vaginal dimensions increase during adolescence, peak at reproductive levels, and decrease sharply after ovariectomy.

- One year after first delivery, the distal vagina gets more compliant, yet this is reversed later in life.

- The lamina propria and muscularis thickened in puberty and in nulliparous. Semi-quantitative collagen assessment demonstrated a lower collagen and higher elastin content after first and multiple deliveries.
Conclusion

- The main changes related to menopause consists in
  - Vaginal atrophy, caused by estrogen loss (genito-urinary syndrome)
  - Shrotening and narrowing of the vagina
  - Lower vaginal contractility
  - Higher collagen and lower elastin density

- Impact on the biomechanical properties of the vaginal wall (> POP)

- The lifetime changes in the Sheep vaginal are similar to those observed in women
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Laser Rejuvenation

- Alternative to first line approach improving vaginal atrophy and dryness by inducing
  - New collagen and elastic fiber production
  - Neovascularisation
  - Improvement of vaginal tissue trophism in general

- New concept « adapted » from results in dermatology or cosmetic surgery
Aim of laser rejuvenation

- To revitalize and restore elasticity and moisture of the vaginal mucosa
  - Topic estrogens
  - Pelvic floor muscles training
  - Hyaluronic acids...

- Via « non invasive » energy based system
Laser rejuvenation

- Plethora of energy-based devices...
  - Laser CO2
    - Monalisa, Femilift, Alma
  - Erbium (YAG)
  - Radiofrequency

...Paucity of well conduct clinical studies...
Laser rejuvenation

- Histological changes in the vaginal wall
Laser rejuvenation

- Histological changes in the vaginal wall

**Sham-controlled study on the short-term effects of Er:YAG laser application in a sheep model for vaginal atrophy**

Lucie Hympanova MD a,b,c, Rita Rynkevic MSc a,b,c, Marina Gabriela Monteiro Carvalho Mori da Cunha PhD a,b, Chantal Diedrich MD f, Silvia Blacher g, Laurent De Landsheere, MD, PhD h, As.prof. Ladislav Krofta c, prof. Jan-Paul Roovers f, prof. Jan Deprest a,b,d

**Conclusions:** Vaginal epithelial thickness sixty days after ovariectomy fell within the reported range of premenopausal sheep (40-180μm). Vaginal Er:YAG laser application was feasible and had some effects on the short term, i.e. higher pH and thicker epithelium compared to sham. Experiments will be completed with invasive reference baseline measurements and a longer interval between ovariectomy and laser application.
Laser rejuvenation

Review

Laser therapy for the genitourinary syndrome of menopause. A systematic review and meta-analysis

Eleni Pitsouni1, Themos Grigoriadis2, Matthew E. Falagas3,4,5, Stefano Salvatore6, Stavros Athanasiou5

1) Department of Obstetrics and Gynecology, "Alexandra" Hospital, National and Kapodistrian University of Athens, Greece
2) AFS Institute of Biomedical Sciences (AFS), Athens, Greece
3) Department of Internal Medicine, Henry-Duane Hospital Center, Athens, Greece
4) Department of Medicine, Tufts University School of Medicine, Boston, MA, USA
5) Obstetrics and Gynecology Unit, Vita-Salute San Raffaele University, IRCCS San Raffaele Hospital, Milan, Italy
6) Obstetrics Unit, Vita-Salute San Raffaele University, IRCCS San Raffaele Hospital, Milan, Italy

and measurements of the effect of laser therapy on the local pathophysiology improved significantly. In conclusion, laser therapy for postmenopausal women with GSM appears promising. It may reduce symptom severity, improve quality of life of postmenopausal women and restore the vaginal mucosa to premenopausal status. However, the quality of the body of evidence is “low” or “very low” and, thus, evidence-based modification of current clinical practice cannot be suggested.
Laser rejuvenation

Conclusion

- Gain in interest of laser therapy as a non-hormonal treatment for GSM

- Laser therapy alleviate vaginal dryness, dyspareunia (and urinary symptoms ?)

- More well-conducted morphometric studies are needed to assess the changes related to laser therapy

- RCT are needed to compare laser with other therapies, as well as the duration of therapeutic effects or repeat applications
Conclusion

And To Avoid...

Designer Pussies?

Vaginal Rejuvenation
 Aimed at women who complain of feeling too loose to enjoy sex; it involves tightening the vagina.
$7,000+

Labiaplasty
This can reduce the size of the inner or outer labia and even out asymmetrical lips.
$6,000

The G-Shot
It increases the size of the G-spot area via a collagen injection; this supposedly intensifies sensation.
$1,550

Watch out fellas don’t be fooled