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# ESTROGEN ENHANCES TENSILE STRENGTH, COLLAGEN CONTENT, AND CYSTATIN-C EXPRESSION IN THE RHESUS MACAQUE VAGINA

**Aims of Study**

Surgeons have a strong clinical bias that estrogen improves the mechanical integrity of the pelvic floor tissues. Collagen is the major determinant of mechanical strength in biologic structures, and functional estrogen receptors are present in the fibroblasts of pelvic floor connective tissues. In an animal model, we can control the major variables affecting prolapse--age and parity--and isolate the effects of hormonal treatment.

In this study, we tested the effect of estradiol on the biomechanical properties and collagen content of the Rhesus macaque vagina. In addition, we examined the effect of estradiol on the expression of genes associated with the extracellular matrix, and their localization within the specific tissues of the vaginal wall.

**Methods**

Sixteen nulliparous, young adult, female macaques underwent oophorectomy. Six animals were treated with an estradiol implant (serum estradiol 33-72 picograms/ml) for 5 months, 7 were estrogen deprived for 5 months, and 3 were estrogen deprived for 14 months. There was no difference between long and short-term hormone withdrawal so data from these two groups were pooled.

Longitudinal strips of full thickness anterior vaginal wall were cut in a standardized “dog bone” shape. Uniaxial tensile failure load and stiffness were measured on an Instron tensiometer. A load displacement rate of 20 mm/min was used, and deflection was measured by optical capture of markers sutured to the specimens. The tissue dimensions were measured from digital photographs of the gross specimens and by image analysis of histologic specimens.

Collagen was measured in samples of the densely ligamentous paravaginal attachment. The amount of collagen was determined by hydroxyproline assay and expressed as a ratio (mg hydroxyproline/mg total protein).

Samples of vaginal wall were frozen for isolation of total RNA. Total RNA was analyzed for 96 ECM gene transcripts on a commercially prepared cDNA array (SuperArray Inc).

Histologic cryosections were used to localize mRNA expression by in situ hybridization (ISH) and protein expression by immunocytochemistry (ICC). Rhesus specific Cystatin-C riboprobes were used for ISH, and anti-human Cystatin-C antibodies were used for ICC.

**Results**

Failure load, a measure of tensile strength, was greater in estradiol treated animals than in deprived animals. Stiffness was also greater in estradiol treated animals, but this was not statistically significant. Estradiol treatment was associated with an increase in collagen (hydroxyproline content) in the dense connective tissue of the paravaginal ligament. Vaginal wall thickness increased with estradiol therapy. The thickness of the fibromuscular layer increased 1.4 fold, and the epithelial layer, 4 fold, under the influence of estradiol ($p<0.05$).

| | **Estradiol treated** | **Estrogen deficient** | ***p, Student's t*** |
|---|---|---|---|
| Failure Load (Newtons) | 13.6 + SE 2.2 | 8.7 N + SE 1.1 | 0.05 |
| Stiffness (Newtons/mm) | 11.2 + SE 4.3 | 7.0 + SE 2.5 | 0.42 |
| Hydroxyproline content | 0.0089 + SE 0.002 | 0.0082 + SE 0.002 | <0.05 |
| Vaginal wall thickness (mm) | 2.9 + SE 0.2 | 1.5 + SE 0.1 | <0.0001 |

Estradiol treatment resulted in significantly upregulated transcripts of the cysteine protease inhibitor, Cystatin-C, 10-20 fold above the level in untreated controls ($p=0.05$) Cystatin-C upregulation by estradiol, both mRNA and protein expression, was primarily localized in the vaginal fibroblasts and smooth muscle cells. Cystatin-C was highly expressed in the stratified squamous epithelium, but this did not vary with hormone treatment.

**Conclusions**

Estrogen increases the tensile strength of the vaginal wall. This increase in tensile strength is likely mediated by both the increased thickness of the vaginal wall and the increase in the amount of collagen. Estrogens also elevate the cysteine protease inhibitor, Cystatin-C. Cysteine proteases have been implicated in collagen and elastin degradation in other systems, including bone resorption. Therefore, by elevating Cystatin-C, estrogens may inhibit proteolysis of extracellular matrix components and thereby contribute to increased tensile strength of the pelvic floor connective tissues. Human studies are needed to assess the importance of this biologic change in the etiology of human pelvic organ prolapse.

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