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Effects of delivery and oophorectomy on urethral collagen: An experimental study


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Abstract

Objective: Stereological evaluation of the concentration of type I and III collagen fibers in the urethral tissue of rats subjected to simulated labor and oophorectomy. To compare the concentrations of collagen between oophorectomized and non-oophorectomized rats.

Materials and methods: Sixty adult Wistar rats were divided into six groups. A group made up of virgin rats was used as control group and another group was made up of oophorectomized rats. Two groups underwent vaginal distention for 30 and 120 min, respectively. The two other groups were subjected to the same distension periods, followed by oophorectomy. Sixty days later, euthanasia and removal of urethral tissue was carried out for stereological analysis of type I and III collagen after staining with hematoxylin and eosin and picrosirius red.

Results: A decrease in estrogen levels was observed in the oophorectomized rats. There was a reduction of type III collagen in the oophorectomized control group compared to the control group when analyzed independently. No significant differences were observed among the other groups. Type I collagen decreased in all groups compared to the control group. However, in the prolonged vaginal distension and oophorectomy group, these fibers increased.

Conclusion: In normal rats, simulation of labor does not alter the collagen III levels. In hypoestrogenic rats, the concentration of collagen type I and III decreased, except in those undergoing prolonged labor simulation in which collagen I increased.

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Introduction

Stress urinary incontinence (SUI) is a problem that affects about 25 million women in the United States, and statistics show that about 30–40% of women will experience some degree of incontinence throughout their life.\(^1,2\) Despite its high prevalence and being evaluated by various groups and countless ways, the SUI pathophysiology is not yet fully understood.

It showed a direct relation between factors such as increased body mass index (BMI) in pregnancy, multiparity, vaginal delivery, the second stage of prolonged labor, and episiotomy with pelvic floor disorders.\(^3\) However, there is no consensus on whether pregnancy or vaginal delivery is responsible for the morbidity rates in these structures.\(^4,5\)

Labor was associated with pudendal nerve injury, partial denervation of the pelvic floor, reduction of urethral pressure parameters, and alterations in innervation and in the pubococcygeus portion of the levator ani muscle.\(^6\) These changes, demonstrated through electromyography, would be the main causes of the pathogenesis of the SUI, and fecal incontinence.\(^7,8\) On the other hand, studies related hormone deprivation with morphological alterations of the bladder and the urethra, greater prevalence of urinary symptoms and urodynamic changes.\(^7,8\) However, women with postpartum SUI tend to recover; the nulliparous ones also have SUI, and significant electrobioelectrical alterations after vaginal delivery have not been demonstrated.\(^9,10\)

The continence mechanism involves the summation of various factors such as the anatomical topography of the structures involved, the vascular and muscular periurethral components, the pelvic musculature, and innervation thereof. Its structural framework consists of muscle fibers and extracellular matrix, which through its components confer carrying capacity, capacity of resistance to effort, and return to the normal form.\(^11,12\) Among them, the elastic, collagen, elaunline, and oxitalamic fibers; the first confer elasticity and the second resistance.\(^12,13\)

Several studies analyzed the alterations of collagen and its possible relation to the SUI evaluating symptomatic women and in different hormonal status; however, the results were different. In them, there has been a deficit of these fibers in incontinent patients, although it was postulated that an increase in collagen could provide greater stiffness to the extracellular matrix and cause failure in mechanical function.\(^14\) This created the need to develop pilot studies using quantitative methods which reliably express the structural changes in the different tissues, with the aim of relating labor, collagen, and the changes resulting from hormonal deprivation with the SUI. Given the diversity of methods that seek to meet this condition, stereology is a very useful alternative to obtain quantitative information efficiently, without bias or systematic error, making it a good choice for the quantification of collagen fibers. This science aims to determine three-dimensional quantitative parameters of anatomical structures from two-dimensional cuts; it uses geometric and statistical principles on data obtained from optic and electron microscopy.\(^15\)

This experimental work assesses the effect of simulated labor and oophorectomy on type I and III collagen fibers in the urethral tissue of rats through the stereological study of these structures. The consequences of the isolated hormone deficiency are also taken into consideration, for which differences in collagen concentrations between oophorectomized and non-oophorectomized rats are analyzed.

Materials and methods

In this study, which was approved by the Ethics Committee on Animal Testing of the Biological Sciences Sector of the Federal University of Paraná, 60 Wistar adult rats, with an
average age of 120 days, whose weight ranged between 200 and 250 g, were used divided into 6 groups:

3. Group C: 10 rats undergoing vaginal distension for 30 min.
4. Group D: 10 rats undergoing vaginal distension for 30 min and subsequent oophorectomy.
5. Group E: 10 rats undergoing vaginal distension for 120 min.
6. Group F: 10 rats undergoing vaginal distension for 120 min and subsequent oophorectomy.

For the trauma simulation, the rats were anesthetized with a combination of xylazine (5 mg/kg) and ketamine (100 mg/kg) intraperitoneally. After induction of anesthesia, a 10 Fr Foley catheter was introduced into the vagina and the balloon was filled with 3 ml saline to reach a diameter of 0.9 cm. The animals positioned on the edge of the table, with the lower legs on the abdomen remained with the intravaginal balloon during the time determined for each group. Once this period was completed, the catheter was removed with the insufflated balloon (Fig. 1).

The oophorectomy was performed immediately after vaginal distension through an anterior incision. During the first postoperative day, the animals received intramuscular analgesics (dipyrone) and associated to hydration.

The dosage of estradiol was performed by means of radioimmunoassay using an anti-estrogen polyclonal antibody in feces collected during the 24h prior to euthanasia and processed according to the technique described by Touma and Palme.

Completed 60 days after the procedure, euthanasia was performed in the rats using thiopental sodium intraperitoneally, and the bladder, the urethra, the vagina and the pelvic floor muscles were immediately resected en bloc.

Then, the urethral and periurethral tissue was dissected. The urethra was sectioned longitudinally, the samples were processed and stained with the hematoxylin-eosin method and the picrosiris red.

The stereological analysis of type I and type III collagen fibers was performed in random cuts and uniformly isotropic, applying the orientator method in histological slides stained with the trichrome histochemical technique of picrosiris and Olympus BX50 microscope with image capture 3CCD pro-series camera. Through this method, the thickest collagen fibers, strongly birefringent, are colored in shades of orange, yellow, and red, corresponding to type I collagen. The finest and most scattered fibers, weakly birefringent, are colored in green, corresponding to type III collagen. For the histomorphological and histomorphometric analysis, the image capture program Imageproplus version 4.5 of Cybernetics (Fig. 2) was also used.

For the analysis in progressive sample of this paper, the orientator method was applied; we conducted a randomization of 5 rats per group, and 5 histological fields of each urethra randomly selected were analyzed, in total 25 fields per group. In these sectors, type I and III collagens stained with picrosiris under polarized light microscopy at 400× magnification were quantitatively analyzed. The images were captured with an Olympus DP71 camera and exported to a Sony Trinitron monitor, frozen and digitized by an Oculux TCX (Coreco) digitizer plaque. All the images were evaluated with the Image Pro-Plus application for Windows on a Pentium III computer. The obtained values were converted into percentages (%) for obtaining the relative frequency.

The data were described using mean and standard deviation values. The distribution thereof, in terms of normality, was tested through the Kolmogorov–Smirnov test. The measures of the oophorectomized and non-oophorectomized groups who passed the normal distribution test were studied with Student’s “t” test or the nonparametric Mann–Whitney test.

Figure 1  CT image where the Foley catheter balloon insufflated into the vagina of a rat to simulate labor can be observed.

Figure 2  Collagen fibers after staining with picrosiris trichrome histochemical technique, observed under polarized light.
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Table 1 Analysis of the level of estrogen (oophorectomized and non-oophorectomized rats).

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of estrogen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Oophorectomized</td>
<td>30</td>
<td>217.1</td>
<td>75.6</td>
</tr>
<tr>
<td>Non-oophorectomized</td>
<td>28</td>
<td>371.7</td>
<td>170.0</td>
</tr>
<tr>
<td>p1 value*</td>
<td>0.0002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2 value**</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p1: Student’s ‘t’ test/Mann–Whitney test. **p2: Kruskal–Wallis test/ANOVA.

For the study of the measures in all the assessed groups, we used, when it was significant, the ANOVA method followed by Tukey’s multiple comparisons. For the data without normal distribution, the Kruskal–Wallis test was used followed, when it was significant, by the Mann–Whitney test. The assumed significance level was 5%, and the SAS version 9.1 software was used for the analysis.

Results

During the experiment, two rats died. When analyzing the weight of the rats in the different groups, there was no statistical difference either between oophorectomized and non-oophorectomized rats or those undergoing vaginal distension.

There was a statistically significant reduction in the level of estrogen of the oophorectomized rats compared to the non-oophorectomized ones (Table 1). But in the study of multiple comparisons this difference was not significant between the controls and the groups undergoing vaginal distension for 30 min.

After the stereological analysis, there was a reduction of the amount of type III collagen fibers in the periurethral tissue of the oophorectomized animals compared to the control group. There were no significant differences in the other comparisons (Table 2).

With regard to type I collagen, it was found to be reduced in all groups in relation to the control, except in the rats undergoing vaginal distension for 120 min followed by oophorectomy. In this group, there was an increase of these fibers by analyzing only the oophorectomized groups (Table 3).

Table 2 Analysis of type III collagen fibers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Type III collagen</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>Oophorectomized</td>
<td>75</td>
<td>25.2</td>
<td>21.8</td>
</tr>
<tr>
<td>Non-oophorectomized</td>
<td>75</td>
<td>33.9</td>
<td>23.0</td>
</tr>
<tr>
<td>p1 value: 0.0108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>31.3</td>
<td>17.3</td>
</tr>
<tr>
<td>Oophorectomized</td>
<td>25</td>
<td>11.5</td>
<td>11.2</td>
</tr>
<tr>
<td>p2 value: 0.0003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Discussion

The development of experimental studies for the assessment of the SUI arises from the need to clarify their pathophysiology and to test therapeutic options in a controlled way, establishing cause and effect relations. The first model was described by Lin, who proposed the vaginal distension in animals through the introduction of a 10 Fr Foley catheter insufflating the balloon with 2.5 ml of water for 4 h. In this study, a functional, biochemical, and histopathological assessment was carried out observing a decrease in smooth and striated muscle fibers in the urethra of incontinent rats, as well as a reduction in the lymph node plexuses in the vaginal wall. In line with Lin, other authors developed similar studies, checked various aspects of the injuries caused by the labor simulation and tried to elucidate the pathophysiology of the SUI.

Regarding the way to distend the vagina, we decided to use a 10 Fr Foley catheter and insufflated balloon with 3 ml saline, as the 0.9 cm diameter reached is twice the cephalic diameter of a neonate rat.

The pathological changes after distension include degeneration of nerve bundles, the progressive increase of the dysfunction between urethral muscle fibers and inflammatory disorders.

When trying to assess the effects of hormone deprivation associated to labor injuries, precedent protocols associated bilateral oophorectomy to vaginal distension, observing a reduction of caveloine levels and an increase in the rate of apoptosis in the surface layers of the urethra in groups subjected to the double procedure.

The collagen exerts an important activity in the composition and function of the urogenital system. There are about
Table 3  Analysis of type I collagen fibers.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oophorectomized</td>
<td>75</td>
<td>36.4</td>
<td>23.1</td>
<td>30.3</td>
</tr>
<tr>
<td>Non-oophorectomized</td>
<td>75</td>
<td>36.1</td>
<td>22.3</td>
<td>38.8</td>
</tr>
<tr>
<td>p1 value: 0.8117</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>47.6</td>
<td>21.6</td>
<td>47.6</td>
</tr>
<tr>
<td>Oophorectomized control</td>
<td>25</td>
<td>32.0</td>
<td>23.8</td>
<td>22.1</td>
</tr>
<tr>
<td>Vaginal distension 30 min</td>
<td>25</td>
<td>26.3</td>
<td>20.4</td>
<td>17.2</td>
</tr>
<tr>
<td>Vaginal distension 120 min</td>
<td>25</td>
<td>34.4</td>
<td>20.3</td>
<td>31.5</td>
</tr>
<tr>
<td>Oophorectomy + vaginal distension 30 min</td>
<td>25</td>
<td>30.7</td>
<td>21.4</td>
<td>34.0</td>
</tr>
<tr>
<td>Oophorectomy + vaginal distension 120 min</td>
<td>25</td>
<td>46.4</td>
<td>21.4</td>
<td>50.1</td>
</tr>
<tr>
<td>p2 value**: 0.0008</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p1: Mann-Whitney test; **p2: Kruskal-Wallis test.

19 types of collagen known, type I and type III collagens being of primary importance in the urogenital tract. Type I collagen is the most abundant in the skin, tendons, ligaments, and bone, corresponding to 80–90% of the total collagen in the body. Type III collagen has a similar distribution, but while type I is related to the strength and elasticity, type III relates to the support. The production and variation between the different types of collagen are regulated by various factors, among them the action of the estrogens. Both the hormonal influence and the role of the collagen in the urinary system have already been discussed, and some experimental studies analyzed it by means of castration protocols followed by replacement.

In this study, different groups of virgin rats underwent vaginal distension and bilateral oophorectomy, finding a statistically significant difference in hormone levels when comparing the groups of oophorectomized rats with the non-oophorectomized ones. However, when comparing the virgin control group to the oophorectomized control and the groups undergoing vaginal distension for 30 min, the difference was not significant. This situation could be due to increased estrogen concentration in feces, which were dehydrated at the time of their collection and the pseudocyesis mechanism generated by vaginal distension.

For the analysis of the collagen fibers, countless studies provide different forms of assessment, such as immunohistochemistry, electron microscopy, electrophoresis, hydroxyproline dosage, or western blot. In this study, we used stereology, a method that has a satisfactory credibility profile for quantification of collagen fibers, determining three-dimensional information from two-dimensional cuts.

Type III collagen fibers maintained their concentration in the normoestrogenic groups, but there was a reduction thereof in the oophorectomized groups. This agrees with the findings of Rizk, who observed an alteration of the proportion collagen I/collagen III with respect to type III collagen function in oophorectomized rats receiving hormone replacement therapy. Although Augusburger did not find the difference in the levels of type I and III collagen fibers in oophorectomized bitches.

As for the type I collagen, we observe a decrease of these fibers in all the groups when comparing it to the control group, except in the prolonged vaginal distension group followed by oophorectomy. Rizk also verified a reduction of type I fibers in oophorectomized groups when compared with the control group, observing the same in both young and old rats. However, these alterations in the collagen associated with hormonal deprivation were discarded by Chen and Falconer.

On the other hand, there was an increase in type I fibers in those undergoing prolonged vaginal distension and oophorectomized, although the levels did not reach those of the control group. Thus, it can be deducted that the vaginal trauma may induce an increase of the amount of such fibers when associated with hypoestrogenism.

**Conclusion**

This study allows us to conclude that the simulation of labor does not alter the levels of type III collagen in normoestrogenic rats. Estrogen deficiency is associated with a reduction of type I and III collagen. However, prolonged trauma under hypoestrogenism conditions is associated with increased collagen I, which would generate a less elastic repair.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**

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