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ORIGINAL ARTICLE

Concentration of elastic system fibers in the corpus cavernosum, corpus spongiosum, and tunica albuginea in the rabbit penis

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The corpus cavernosum (CC) extracellular matrix is essential for normal penile erection and is implicated in erectile dysfunction. Although investigations of these issues have used the rabbit CC, organization of its components is not well known to date. We characterized and quantified the volumetric density (Vv) of the elastic system fibers in the corpus spongiosum (CS), CC and tunica albuginea (TA) of the rabbit penis. Adult New Zealand rabbits ($n = 10$) were used. The penile mid-shaft fragments were fixed with 4% phosphate-buffered formalin solution and/or Bouin's liquid for 24–48 h, and processed using standard histological techniques. The sections were stained with Weigert's Fucsin–Resorcin with previous oxidation. The elastic system fibers Vv (%) was determined in 25 random fields of each fragment, using the M-42 test grid. The histochemical methods detected elastic system fibers in CS, CC and TA of all animals. The Vv of elastic fibers average was 25.03 ± 2.0% for CC, 32.23 ± 1.41% for CS and 22.38 ± 3.61% for TA. Results for CC and CS were not significantly different. The great amount of elastic fibers distribution beneath the endothelium suggest that these fibers may have an important role in the erection process in rabbits. The present data should therefore provide important information for devising experiments and interpreting results when using the rabbit penis as a model for penile dysfunctions, especially when making comparisons with humans.
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Introduction

The general understanding of the morphological changes and physiology of penile erection is obtained through several studies considering different animal models[1–3] such as rats,[1,4,5] domestic animals, primates[3,6–8] and rabbits.[2,9]

Morphological and quantitative data concerning rabbit penis are still scarce, and there is need for more information, mainly because this animal is frequently used as a model for erectile dysfunction.[2,9–11]

Although the gross anatomy between rabbits and other animals is different, histological structural elements are similar in mammals, but with special characteristics for each species.[12]

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Elastic and collagen fibers are important penile constituents and maintain the penile structure during erection, and allow adequate resistance during the return to the nonerect state.[13–16]

The literature available has been focusing on the general morphology of the rabbit penis.[17,18] Therefore, no information is available on qualitative or quantitative connective tissue elements. The purpose of this study is to gain a better understanding of the rabbit penis using morphometrical analysis of the elastic fibers in the corpus spongiosum (CS), corpus cavernosum (CC) and tunica albuginea (TA).

Materials and methods

Animals

The ethical committee of the State University of Rio de Janeiro approved the research protocol. Adult New Zealand rabbits ($N = 10$) were obtained from a commercial abattoir. After the rabbit was killed, the penis was removed and immediately fixed in 4% phosphate-buffered formalin solution for 24–48 h. Afterwards, penile mid-shaft segments were pro-

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cessed according to the standard histological techniques for paraffin embedding.

Quantitative analysis

From each penis, five different sections were selected from five fragments. Then, five random fields were evaluated from each section. There were, therefore, 25 test areas from each penis. For the stereological analysis, 5 μm sections were stained with Weigert's Fucsin–Resorcin. This stain was used after oxidation to detect oxitalan, elaunin and elastic fibers,[19] so as to be able to quantify these elastic system components.The data were expressed as volumetric densities (Vv%). The analyzed fields were digitized with $\times 400$ final magnification using a video camera coupled to a light microscope. The selected histological areas were then quantified using M42 test-grid system on the digitized fields on a color monitor screen. From stereological principles in isotropic tissue, the distribution area of a specific structure, as determined on a two-dimensional section of a structure, is proportional to the volume distribution of the structure.[1,20,21] The volume density of the histological components was calculated by Vv = Pp/Pt, where Vv is the volume density, p is the tissue component under consideration (elastic fibers), Pp is the number of test points associated with p, and Pt is the number of points of the test system. Line and point probes are obtained in a field by superimposing a grid consisting of an arrangement of lines and points. The stereological methods have been described in detail elsewhere.[21–24]

Statistical analysis

The data were analyzed in the software Graphpad Instat (Graphpad) to verify the Gaussian distribution. To compare the quantitative data of CS and CC, the Student's *t*-test was used ($P<0.05$ was considered significant).

Results

The rabbit has a vascular penis, which contains two erectile structures: a supero-lateral CC and the ventral CS that surrounds the penile urethra (Figure 1). Both structures were covered by a dense capsule of connective tissue, the TA, which projects intracavernosal pillars or septa, mainly in the CC (Figure 2).

The histochemical analysis confirmed the presence of elastic system fibers in CC (Figure 3), CS (Figure 4) and TA of all specimens observed (Figure 5).

An irregular elastic fiber network was distributed throughout the penis (Figure 6). Connective tissue elements, mainly elastic system fiber, were abundant. In the CS, the amount of elastic fiber was greater (32.3%) than in the CC (25.1%); however, this difference was not significant. These fibers often shown a tortuous profile, and surrounded sinusoids in the CS (Figure 6). The Vv density of elastic system fibers in the TA was 22.4%. The quantitative data on the elastic system fibers are summarized in Table 1.

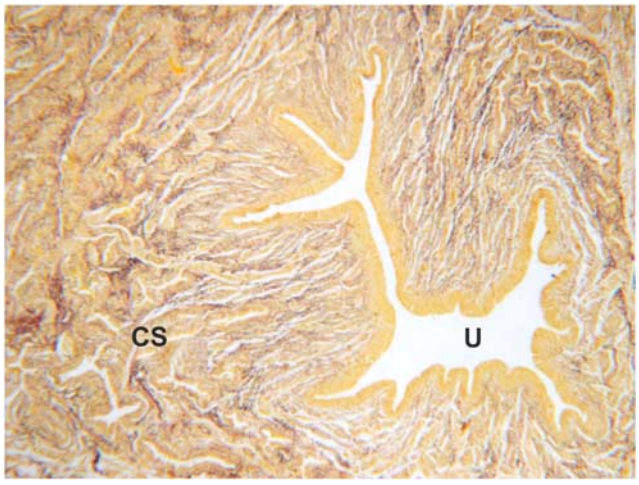

Figure 1 Cross-section (transversal) of rabbit penis. U = urethra, CS = *corpus spongiosum*, Weigert's Resorcin–Fucsin, $\times 40$.

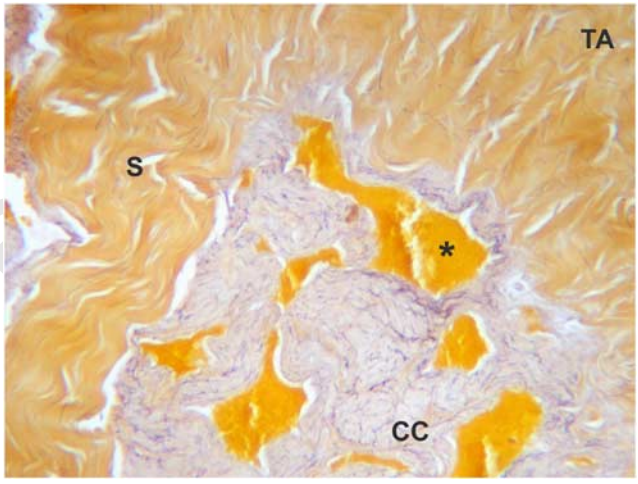

Figure 2 Histological section of *corpus cavernosum* (CC). Note the tunica albuginea (TA), intracavernosal septum (S), Weigert's Resorcin–Fucsin stain, $\times 100$.

Discussion

Most of the studies attempting to quantify linear structures make use of areal density, mainly since the advent of computer-aided image analysis programs.[25–28] These programs use the color property of

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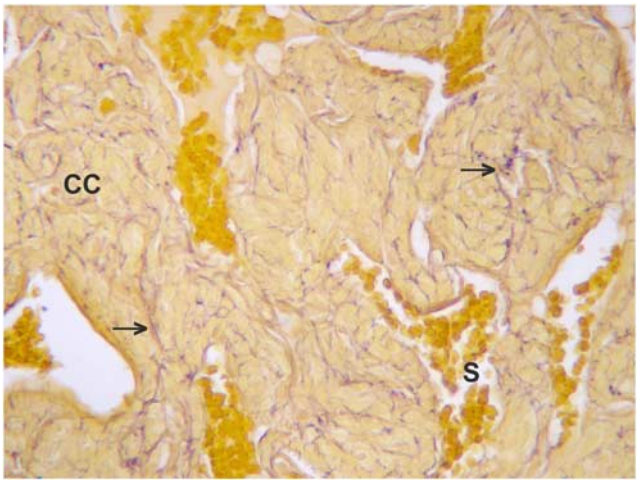

Figure 3 Histological section of the *corpus cavernosum* containing a network of elastic (arrow) fibers, Weigert's Resorcin–Fucsin stain, × 200.

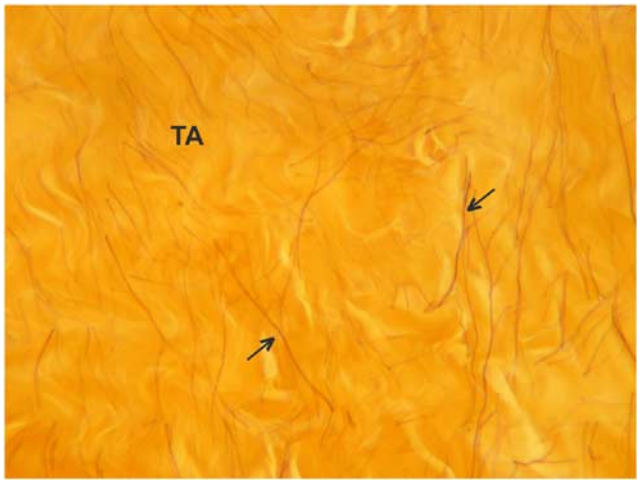

Figure 5 Tunic albuginea displaying the meshwork structure of elastic fibers (*), Weigert's Resorcin–Fucsin, × 200.

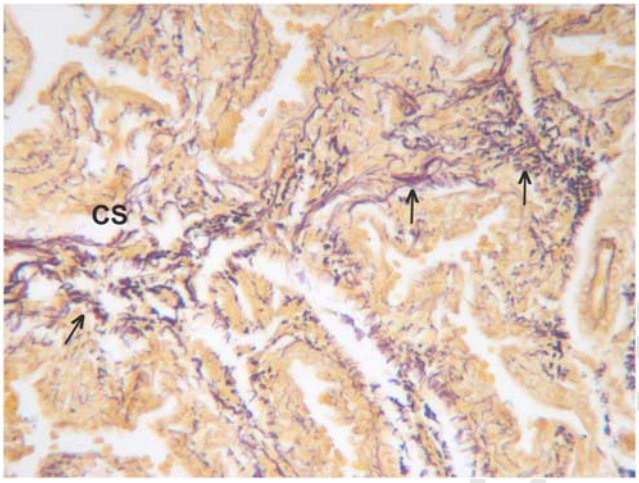

Figure 4 Volumetric density of elastic fibers (arrows) is more abundant in the *corpus spongiosum* than in the *corpus spongiosum*, Weigert's Resorcin–Fucsin, × 200.

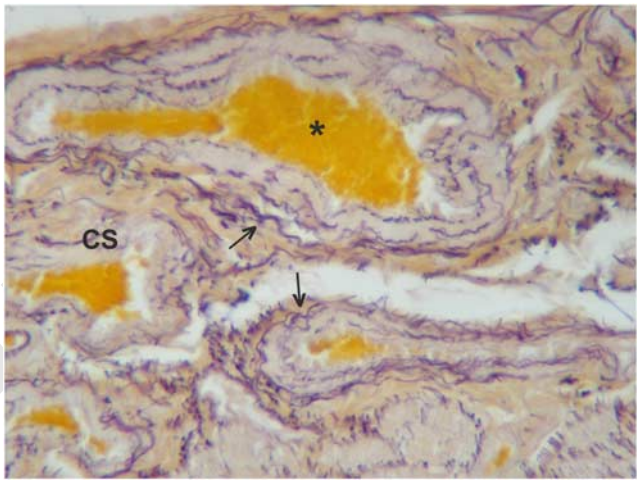

Figure 6 Elastic fibers surrounding sinusoids (*) in the *corpus spongiosum*, Weigert's Resorcin–Fucsin stain, × 200.

Table 1

Parameter	*Corpus spongiosum*	*Corpus cavernosum*	*Tunica albuginea*
Mean	32.3%	25.1%	22.4%
s.d. (±)	1.4	2.0	3.6

the elements (pixels) of an image to determine a threshold level for inclusion. This is a rapid procedure, but for most linear structures it is not the appropriate tool because the most significant increase in these structures is in their length and not in their volume. Moreover, when very thin linear structures are under analysis and the contrast between the fibers and the background is low, as is the case with elastic systems fibers, the error introduced using color intensity as the measurement method is too high, thus making it impossible to use the volume density as a reliable method of study.[29]

The stereological method has been used to quantify urogenital tissues, for example, to determine the Vv of elastic system fibers in the rat penis,[1] wild boar penis[30] and human penis in the fetal period[16,39] and other extracellular matrix elements of the human prostate.[20,31] The stereological method has been recommended by several authors[21–24] to avoid the bias that frequently occurs during automatic or semiautomatic computerized image analyses, which could over- or underestimate the analyzed structures.[28]

Normal tissue development and maintenance depend on the intercellular and cell–matrix interac-

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tions.[32,33] The fiber locations and arrangement are related to their different functionality, which reflects local tissue mechanical properties. The elastic system is formed by three types of fibers, that is, oxytalan, elaunin and elastic. The oxytalan fibers are formed exclusively by microfibrils, the elaunin fibers by microfibrils and patches of amorphous material (elastin), and the elastic fibers by a large amount of elastin with fibrils.[16,34] These fibers are characterized by extension and elastic recoil qualities.[32,34–36]

Tissues that are constantly submitted to tensile strength are rich in elastic fibers like the penis.[33,34] Intumescence and detumescence require a complex structure, and are closely associated with connective tissue distribution, mainly elastic fibers and collagen bundles that also have a stretching function during erection.[15,28]

Our study showed that the elastic system fibers were abundant in the CS of the rabbit, demonstrating a greater Vv in contrast to the CC and TA.

In mammals, the classification of different penis types is based on erectile or connective tissue.[12,18,37] In animals, with vascular penis, for example, rabbit, or in man, erection is a consequence of increase in size and hardening of the organ. In animals with a fibroelastic penis, the erection is essentially a result of length increasing, with the penis emerging from the prepuce due to sigmoid flexure straightening.[12,18,37,38] The fibroelastic penis is typical in domestic pigs, cattle, but is without intumescence, that is, there is volume increase in contrast to the human penis, which is a vascular type.[17] Thus, sigmoid flexure in these animals is important for penile straightening.

Although elastic fibers in the CS and the CC provide elasticity and reliance critical properties, few quantification data on the fiber distributions are available for the mammalian penis.[1,30]

Elastic fibers are the major extracellular matrix components of the human fetal urethra[40] and their Vv in the CS increases in a linear fashion during fetal development (from 5.2% at 15th week to 14.8% at 36th week). In a young male, the elastic fiber Vv is about 19%.[16] In rat CC, Pinheiro *et al.*[1] found 9% of elastic fibers, using the stereologic method, and concluded that the cellular and matricial components of the rat CC differ markedly from those of humans in content and organization. Consequently, inferences and correlations based on physiological and pathological findings derived from experiments that use the rat as an erection model may be misleading if these differences are not considered.

Sattar *et al.*[28] found that the concentration of elastic fibers in intracavernous trabeculae of patients with arteriogenic or venogenic erectile dysfunction was significantly lower than that in a potent man. In this particular study, the light intensity and color contrasting observation on computerized images previous (semiautomatic method) was used.

The present results showed that the New Zealand rabbit penis is a vascular organ with prominent elastic fibers in the CS (Vv = 32.3%) and CC (Vv = 25.1%), as well as in the TA (Vv = 22.4%). The elastic system fibers displayed a more tortuous pattern in the CS than in the CC. As in other mammals, these fibers may have an important role in CS expansion and reliance during voiding urine, erection and ejaculation.[15,16,38]

Although the animals possess similarities to humans with regard to the erectile function, normal or abnormal, comparative researches provide better comprehension of the similarities and differences about morphological structure and erectile function among the different species and in the human.[40] In this study, we observed a larger amount of elastic fibers in the rabbit penis than in human penis components, that is, CC, CS and TA. The literature points out that rabbits possess the morphological characteristics of a vascular penis, and therefore can be compared with humans.[11,12,18,37,38]

Information on elastic fiber quantification in the rabbit penis has not been previously reported. Thus, the present study is the first report on the vascular penis type in this animal species.

Conclusion

In conclusion, we found that the elastic system fibers are one of the major components of the extracellular matrix in the New Zealand rabbit penis. The present data should therefore provide important variables for devising experiments and interpreting results when using the rabbit penis as a model for penile dysfunctions, especially when making comparisons with human tissue.

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