

COMPOSITIONAL CHANGES OF COLLAGEN AND GLYCOSAMINOGLYCANS IN THE TUNICA ALBUGINEA AND CORPUS CAVERNOSUM FROM THE HUMAN PENIS DURING THE FETAL AND POSTNATAL PERIODS

ANA L. BASTOS, FRANCISCO J. B. SAMPAIO AND LUIZ E. M. CARDOSO*

From the Urogenital Research Unit, State University of Rio de Janeiro, Rio de Janeiro, Brazil

ABSTRACT

Purpose: We investigated the composition of collagen and glycosaminoglycans (GAGs) in the corpus cavernosum (CC) and tunica albuginea (TA) of normal human penises.

Materials and Methods: Penises were obtained from a 6-month-old child (group 1), a 2-year-old child (group 2), 18 to 34-year-old adults (group 3), 37 to 53-year-old adults (group 4) and 22 fetuses at 17.2 to 33.3 menstrual weeks (group 5). Total GAG and collagen concentrations were expressed per mg dry tissue and proportions of GAG species were determined by agarose electrophoresis and ion exchange chromatography.

Results: The GAG concentration in group 1 CC and TA was 1.32 and 0.52 $\mu\text{g}/\text{mg}$, respectively, and thereafter it increased noticeably. TA collagen concentration followed a similar pattern. TA had more collagen than CC in groups 3 (mean \pm SD 93.41 ± 6.17 vs 53.77 ± 11.18 $\mu\text{g}/\text{mg}$, $p < 0.001$) and 4 (89.94 ± 5.53 vs 55.39 ± 5.89 $\mu\text{g}/\text{mg}$, $p < 0.01$). In these groups TA and CC differed markedly in the proportion of hyaluronan, heparan sulfate and dermatan sulfate. In TA group 4 had slightly less hyaluronan and more chondroitin sulfate than group 3 but in CC the GAG proportions were similar. Collagen content in the whole fetal penis correlated with gestational age ($r = 0.78$, $p < 0.001$).

Conclusions: Collagen and the GAG concentration in the human penis undergo extensive modifications during development and shortly after birth but from ages 2 to approximately 46 years changes are limited to the proportion of GAG species in TA from older individuals. Reflecting diverse biomechanical roles, the extracellular matrix of CC and TA are markedly different.

KEY WORDS: penis, fetus, extracellular matrix, collagen, glycosaminoglycans

The trabeculae of the corpus cavernosum and tunica albuginea are major penile structures involved in erection.¹ They are made up of endothelial cells, smooth muscle cells^{2–4} and extracellular matrix, of which the main components are collagens,⁵ elastic fibers⁶ and proteoglycans/glycosaminoglycans (GAGs).⁷ Histochemical and immunohistochemical analyses, of which some were associated with morphometry, have characterized structural components in the erectile tissue of human adults^{2,6} and more recently these techniques have been used to investigate connective tissue in the human fetal penis.^{3,4} However, the biochemical composition of human penile extracellular matrix in fetal or postnatal samples is still poorly known, especially with regard to GAG.

Qualitative and quantitative alterations in the corpus cavernosum and tunica albuginea, including those resulting from aging, pathological conditions and social habits such as smoking, have been associated with erectile dysfunction.⁸ Indeed, aging adversely affects the normal function of penile erectile structures, leading to modifications in cellular growth and to remodeling of the extracellular matrix,⁹ although changes in collagen have been reported to be less pronounced.¹⁰ In addition, modifications of extracellular matrix components are often detected in penile samples from

impotent men.^{5,10} However, there are sparse data on proteoglycan composition in penile erectile tissue,⁷ especially in a sample spanning a broad age interval. In addition to providing important information for experiments of the pathophysiology of penile disorders, data on penile extracellular matrix might also be beneficial for investigations of techniques of surgical reconstruction of the penis, especially those using different materials.¹¹

Therefore, in the current study we investigated the biochemical composition of the normal human penis extracellular matrix by analyzing collagen and GAG. We used penises from fetuses at various gestational ages as well as postnatal samples. The latter included specimens from children and adults with the corpus cavernosum and tunica albuginea analyzed separately.

MATERIALS AND METHODS

Preparation of tissue samples. Postnatal samples were obtained from a 6-month-old child, a 2-year-old child and various adults divided into the groups adults I and adults II, in which the approximate age ranges were 18 to 34 and 37 to 53 years, respectively. The actual number of samples and mean age in these latter 2 groups varied according to the experiment and they are indicated when appropriate. Postnatal samples were obtained during autopsy, consisting of the corpus cavernosum and its associated tunica albuginea, which were analyzed separately. These tissue samples were dissected from the mid shaft and did not include the deep cavernous artery.

Submitted for publication May 13, 2004.

Supported by grants from the National Council for Scientific and Technological Development (CNPq), Foundation for Research Support of Rio de Janeiro (FAPERJ) and Foundation for Improvement of Graduate Students (CAPES), Brazil.

* Correspondence: Urogenital Research Unit—UERJ, Av. 28 de Setembro, 87—fundos—FCM—térreo, 20551-030, Rio de Janeiro, RJ, Brazil (FAX: + 55 21-2587-6121; e-mail: lemc@uerj.br).

The 22 fetal penises could not be reliably dissected into the corpus cavernosum and tunica albuginea. Since we wanted to compare fetal and postnatal penises, we took from the former only the external mid third and discarded its skin. This resulted in small tissue samples, which were analyzed entirely and only for collagen. The fetuses died of causes not related to the urogenital tract and they were fresh, macroscopically well preserved and showed no external signs of congenital malformation. Gestational age determined in menstrual weeks using the foot length method¹² was 17.2 to 33.3 weeks.

Immediately after dissection tissue samples were fixed in cold acetone and kept in this fixative for 24 hours at 4°C. Samples were then finely minced and submitted to 2 changes of 24 hours each in 40 ml chloroform, namely methanol (2:1 volume per volume) at room temperature. The solvent was then decanted and after incubation at 60°C for 30 minutes a preparation of dry and defatted penile tissue was obtained and weighed.

GAG extraction, identification and quantitation. Procedures for the extraction, purification, identification and quantitation of GAG were done as described in detail previously.¹³ Briefly, about 15 to 25 mg of dry penile tissue samples were digested with twice crystallized papain (Sigma Chemical Co., St. Louis, Missouri) and free GAG chains in the supernatant were isolated by precipitations with cetylpyridinium chloride and ethanol. The amount of GAG in this preparation was assessed by a hexuronic acid assay and it is expressed in μg hexuronic acid per mg dry defatted tissue. Identification of the different GAG species was accomplished by agarose gel electrophoresis combined with analysis of the susceptibility of individual GAG bands to degradation by chondroitin AC and ABC lyases (Sigma Chemical Co.) and nitrous acid. For the quantitation of individual GAG species total GAG was fractionated by ion exchange chromatography on DEAE-Sephacel columns (Pharmacia, Uppsala, Sweden) equilibrated with 0.05 M sodium acetate buffer, pH 6.0, containing 0.1 M NaCl and eluted with a linear NaCl gradient from 0.1 to 0.9 M in this buffer and at a flow rate of 13 ml per hour. Proportions of hyaluronan, heparan sulfate and dermatan sulfate plus chondroitin sulfate were determined by calculating peak areas in the elution profiles. The individual proportions of dermatan sulfate and chondroitin sulfate were assessed by agarose gel electrophoresis, followed by densitometry of toluidine blue stained bands using Scion Image, version 4.0.2 software (Scion Corp., Frederick, Maryland). Proportions are expressed as a percent of total GAG.

Determination of collagen concentration. The concentration of total collagen in dry preparations of penile tissue was determined by a hydroxyproline assay. Thus, about 10 to 15 mg of tissue sample were hydrolyzed in 6 N HCl,¹⁴ after which hydroxyproline was assayed, as previously described.¹³ Results are expressed as μg hydroxyproline per mg dry defatted tissue.

Statistics. Statistical procedures were done as described previously.¹⁴ Concentrations of total collagen and GAG were compared using the Wilcoxon 2-sample test. The statistical association between fetal age and the penile collagen concentration was investigated by linear regression analysis, followed by the t test for the correlation coefficient. Results are shown as the mean \pm SD with statistical significance considered at $p < 0.05$.

RESULTS

At age 6 months the concentration of total GAG in the corpus cavernosum and tunica albuginea was 1.32 and 0.52 $\mu\text{g}/\text{mg}$, respectively, and thereafter it increased noticeably in postnatal penises (fig. 1). However, in the age groups 2 years, adults I and adults II concentrations did not change significantly. On the other hand, the GAG concentration in the

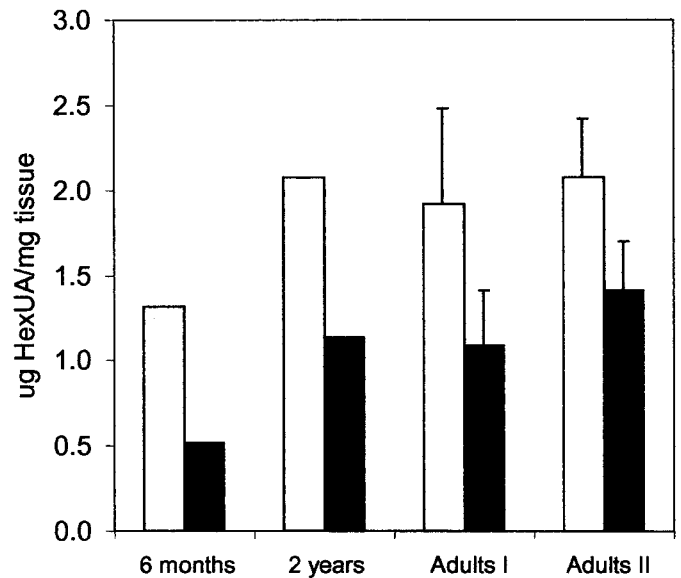


FIG. 1. Total GAG in corpus cavernosum (open bars) and tunica albuginea (black bars) from postnatal penile samples in age groups 6 months (1 subject), 2 years (1 subject), adults I (14 subjects with mean age of 24.50 ± 4.35 years) and adults II (5 subjects with mean age of 43.60 ± 6.69 years) was extracted by papain digestion and precipitation with cetylpyridinium chloride, and quantitated by hexuronic acid (HexUA) assay. Results are shown as μg hexuronic acid per mg dry tissue. GAG concentration in corpus cavernosum was significantly different from that in tunica albuginea in adults I and II (Wilcoxon 2-sample test $p < 0.01$ and 0.02 , respectively). However, there were no significant differences when GAG concentration in corpus cavernosum or tunica albuginea was compared between these age groups. Bars represent mean \pm SD.

corpus cavernosum was significantly higher than that in the tunica albuginea in adults I (1.92 ± 0.56 vs 1.09 ± 0.32 $\mu\text{g}/\text{mg}$, $p < 0.01$) and II (2.07 ± 0.35 vs 1.41 ± 0.29 $\mu\text{g}/\text{mg}$, $p < 0.02$). The collagen concentration in postnatal tunica albuginea followed a similar pattern with homogeneously greater values from age 2 years and thereafter compared with the 6-month-old sample (78.89 $\mu\text{g}/\text{mg}$) (fig. 2). However, in corpora cavernosa the concentration was approximately the same in all age groups studied. Additionally, the collagen concentration in the tunica albuginea was much higher compared with that of the corpora cavernosa in adults I (93.41 ± 6.17 vs 53.77 ± 11.18 $\mu\text{g}/\text{mg}$, $p < 0.001$) and II (89.94 ± 5.53 vs 55.39 ± 5.89 $\mu\text{g}/\text{mg}$, $p < 0.01$).

The relative concentration of individual GAG species in the penis was determined by a combination of ion exchange chromatography and agarose gel electrophoresis using samples from the groups adults I and II (fig. 3). In the tunica albuginea dermatan sulfate was the predominant species, representing approximately 45% of total GAG, whereas heparan sulfate was present in minor amounts (approximately 10%) (fig. 3, A). Furthermore, in this tissue adults II tended to have slightly less hyaluronan ($20.4\% \pm 11.9\%$ vs $26.7\% \pm 5.2\%$) and more chondroitin sulfate ($26.0\% \pm 10.7\%$ vs $17.5\% \pm 6.7\%$) than adults I. However, in the corpus cavernosum the overall proportions of GAG species were similar in the 2 age groups but they differed markedly from those in the tunica albuginea (fig. 3, B). Thus, in addition to hyaluronan, which was the prevailing GAG (approximately 37%), the relative contents of heparan sulfate (approximately 26%) and dermatan sulfate (approximately 19%) were noticeably different in comparison to the corresponding values for tunica albuginea.

Fetal samples consisted of the whole mid third of the penis and they were analyzed for collagen concentration only. Plotting concentration values against fetal age showed a strong and positive correlation ($r = 0.7837$, $p < 0.001$) with values

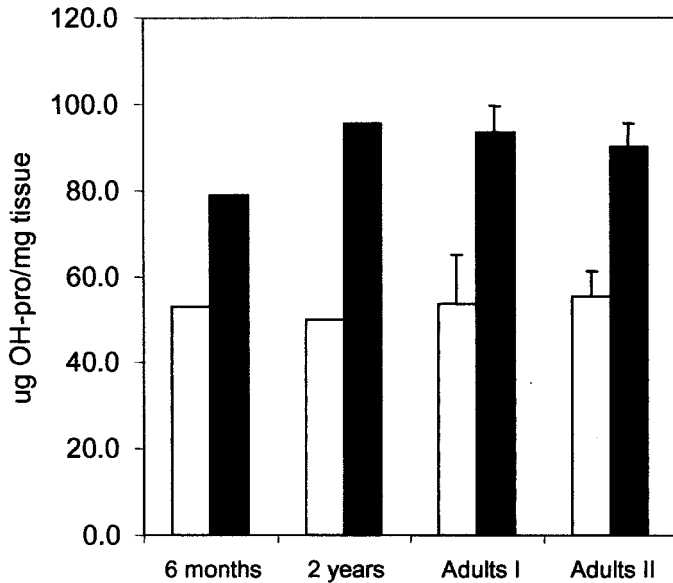


FIG. 2. Total collagen in corpus cavernosum (open bars) and tunica albuginea (black bars) from postnatal penis samples in age groups 6 months (1 subject), 2 years (1 subject), adults I (14 subjects with mean age of 24.50 ± 4.35 years) and adults II (5 subjects with mean age of 43.60 ± 6.69 years). Tissue samples were submitted to acid hydrolysis, followed by hydroxyproline (OH-pro) assay to estimate collagen concentration, which is expressed as μg hydroxyproline per mg dry tissue. Collagen concentration in corpus cavernosum was significantly different from that in tunica albuginea in adults I and II (Wilcoxon 2-sample test p 0.001 and 0.01, respectively). However, there were no significant differences when collagen concentration in corpus cavernosum or tunica albuginea was compared between these age groups. Bars represent mean \pm SD.

almost doubling from gestational ages 17 (approximately 33 $\mu\text{g}/\text{mg}$) to 33 (approximately 56 $\mu\text{g}/\text{mg}$) weeks (fig. 4).

DISCUSSION

Our results in the fetal penis showed that the collagen concentration increased steadily and almost doubled its value from gestational ages 17 to 33 weeks, suggesting that important developmental changes relating to the penile extracellular matrix occur in this period. Similar extracellular matrix remodeling has been found in the human fetal bladder, in which the collagen content also increases with gestational age.¹⁵ Furthermore, if the average postnatal collagen concentration is considered, a 6-month-old individual has a slightly higher value than a fetus at week 33, which indicates that the gradual increase seen during development continues shortly after birth. However, it should be pointed out that collagen as well as GAG concentrations in the corpus cavernosum and tunica albuginea remain unchanged from ages 2 to 36 to 53 years. Thus, the hormonal alterations that markedly affect penile morphology and function with the onset of adolescence have little or no effect on the overall collagen and GAG composition of penile erectile tissues. Consequently these morphological changes should mainly involve the reorganization of cells and/or other extracellular matrix components such as elastic fibers. Indeed, using scanning electron microscopy Shen et al reported that reorganization of cells and elastic fibers occurs in the rat penis as a result of aging.¹⁶

In addition to differences in the concentrations of total collagen and GAG, the extracellular matrix of the corpus cavernosum and tunica albuginea also differed markedly with respect to the relative contents of GAG species. Such differences, which reflect the diverse morphological organizations of these tissues and their distinct roles in erection, have also been described in a report that, however, restricted analyses to GAG in the normal adult tissue.⁷ In that report

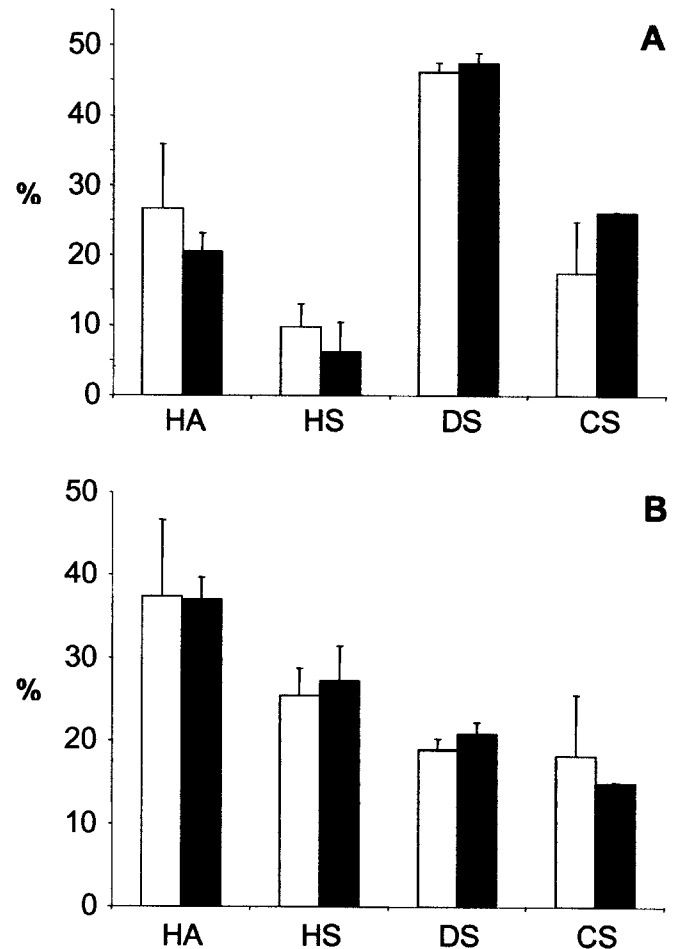


FIG. 3. Relative content of different GAG species in tunica albuginea (A) and corpus cavernosum (B) from human penises in adults I (open bars) and adults II (black bars) age groups. Total GAG preparations were fractionated by ion exchange chromatography on DEAE-Sephacel, which yielded 3 peaks corresponding to hyaluronan (HA), heparan sulfate (HS), and mixture of chondroitin sulfate (CS) and dermatan sulfate (DS). Chondroitin sulfate and dermatan sulfate in last peak were resolved by agarose gel electrophoresis, followed by densitometry of stained bands. Proportion of each GAG was then determined by calculating peak areas in elution and densitometric profiles, and expressed as percent of total GAG. Bars represent mean \pm SD of adults I (4 subjects with mean age of 23.25 ± 2.22 years) and adults II (2 subjects with mean age of 46.00 ± 9.89 years) (A), and adults I (5 subjects with mean age of 25.40 ± 3.21 years) and adults II (2 subjects with mean age of 47.00 ± 8.48 years) (B).

the total GAG concentration, and the relative contents of dermatan sulfate and heparan sulfate in the corpus cavernosum and tunica albuginea are similar to our results. On the other hand, the reported relative content of approximately 30% for hyaluronan in these 2 tissues are at variance with our results, which showed that the corpus cavernosum has almost twice as much of this GAG as the tunica albuginea. In fact, our findings agree with well established data indicating that fibrous, collagen enriched tissues often have much less hyaluronan than looser, more hydrated ones.¹⁷ Because the age groups in the 2 studies were similar, these conspicuously contrasting results might be attributable to the sources of tissue samples since ours were from normal subjects who died of accidents, whereas those in the mentioned report were obtained during surgery for penile cancer. Indeed, *in vitro* studies have shown that hyaluronan synthesis is increased in malignant tumors and metastases.¹⁸ Our results also showed that samples of tunica albuginea from individuals at a mean age of 46 years had slightly different proportions of hyaluronan and chondroitin sulfate compared with

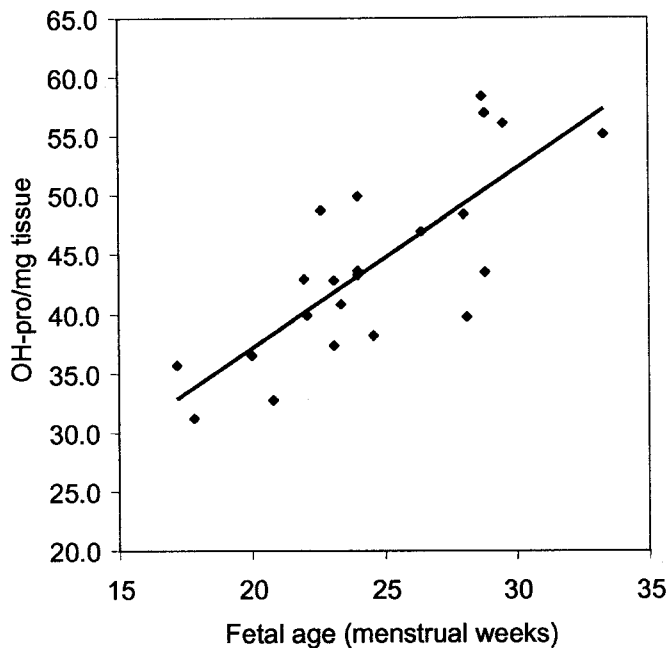


FIG. 4. Total collagen concentration in 22 human fetal penises at different gestational ages. Whole mid third segments from penises were defatted, dried and submitted to acid hydrolysis and hydroxyproline (OH-pro) assay to estimate collagen concentration, expressed as μg hydroxyproline per mg dry tissue. Linear regression and t test for correlation coefficient indicated that fetal age correlated significantly and positively with collagen concentration ($r = 0.7837$, $p = 0.001$).

the group at a mean age of approximately 23 years. Such differences are indicative of stiffer tissue and they might represent an early alteration in the tunica albuginea due to aging. In older individuals other modifications in penile extracellular matrix would then occur, such as the increase in cavernous collagen content.¹⁰

Dermatan sulfate proteoglycans such as decorin are known to sequester growth factors and, thereby, prevent the activation of crucial growth factor receptors, including those present on cavernous smooth muscle cells.¹⁹ Because the corpus cavernosum has a much lower dermatan sulfate content than the tunica albuginea, as shown by our results, conditions that involve enhanced release of growth factors, for example inflammatory responses and hypoxia, would lead to greater smooth muscle cell stimulation in the corpus cavernosum. Thus, outcomes such as smooth muscle cell proliferation, increased matrix molecules expression and fibrosis would be comparatively more pronounced in the corpus cavernosum. Also noteworthy is that, although it is a vascular tissue, the corpus cavernosum has a GAG composition that differs markedly from that of an artery. Thus, its overall GAG concentration and the combined relative contents of chondroitin sulfate plus dermatan sulfate correspond to almost half the values of an artery.¹³ Since particular GAG compositions in the arterial wall are related to lipoprotein retention, smooth muscle cell activation and ultimately to the development of the atheromatous plaque,¹³ one may infer that the corpus cavernosum is likely at lower risk for atherosclerotic disease than a large artery. However, to our knowledge it is not yet possible to ascertain how cavernous proteoglycans are implicated in vasculogenic erectile dysfunction and experiments involving lipoprotein assays in animal models and in humans would have to be performed to address this issue.

The biomechanical properties of a tissue depend directly on the particular composition of its extracellular matrix. For example, in the arterial wall resistance to stretching de-

creases markedly when GAG or collagen is experimentally degraded.²⁰ Our results show that the tunica albuginea and corpus cavernosum, which have different biomechanical roles in the physiology of erection,¹ have distinct extracellular matrices. Therefore, their compositions should be considered in studies of the surgical reconstruction of penile tissues. This might improve the overall efficiency of such techniques, especially when they involve the selection and/or preparation of other materials, for example allograft processed skin or synthetic fibers.¹¹

CONCLUSIONS

The extracellular matrix of the human penis changes noticeably during development and shortly after birth but from ages 2 to approximately 46 years there are only slight modifications in GAG proportions in older individuals. In line with their diverse roles in erection the extracellular matrix composition of the corpus cavernosum and tunica albuginea are markedly different.

REFERENCES

- Gillon, G. and Barnea, O.: Erection mechanism of the penis: a model based analysis. *J Urol*, **168**: 2711, 2002
- Wespes, E., Goes, P. M., Schiffmann, S., Depierreux, M., Vanderhaeghen, J. J. and Schulman, C. C.: Computerized analysis of smooth muscle fibers in potent and impotent patients. *J Urol*, **146**: 1015, 1991
- Sampaio, F. J. B., Pinto, J. L. F., Silva-Costa, W. and Pereira, M. J. S.: Quantitative analysis of smooth muscle fibers in corpus cavernosum of human fetuses. *J Urol*, **159**: 2226, 1998
- Bastos, A. L., Costa, W. S., Cardoso, L. E. and Sampaio, F. J.: Collagen and elastic fibers in the penis of human fetuses at 28 weeks postconception. *Eur Urol*, **36**: 158, 1999
- Raviv, G., Kiss, S., Vanegas, J. P., Petein, M., Danguy, A., Schulman, C. et al: Objective measurement of the different collagen types in the corpus cavernosum of potent and impotent men: an immunohistochemical staining with computerized-image analysis. *World J Urol*, **15**: 50, 1997
- Goldstein, A. M., Meehan, J. P., Morrow, J. W., Buckley, P. A. and Rogers, F. A.: The fibrous skeleton of the corpora cavernosa and its probable function in the mechanism of erection. *Br J Urol*, **57**: 574, 1985
- Goulas, A., Papakostantinou, E., Karakiulakis, G., Mirtsou-Fidani, V., Kalinderis, A. and Hatzichristou, D. G.: Tissue structure-specific distribution of glycosaminoglycans in the human penis. *Int J Biochem Cell Biol*, **32**: 975, 2000
- Atahan, O., Kayigil, O. and Metin, A.: Electrical activity of corpus cavernosum in vasculogenic and non-vasculogenic erectile dysfunction. *Scand J Urol Nephrol*, **31**: 549, 1997
- Montorsi, F., Briganti, A., Salonia, A., Deho, F., Zanni, G., Cestari, A. et al: The ageing male and erectile dysfunction. *BJU Int*, **92**: 516, 2003
- Lin, J. S., Lin, Y. M., Chow, N. H., Wang, S. T. and Sun, Y. N.: Novel image analysis of corpus cavernous tissue in impotent men. *Urology*, **55**: 252, 2000
- Leungwattanakij, S., Bivalacqua, T. J., Yang, D. Y., Hyun, J. S. and Hellstrom, W. J.: Comparison of cadaveric pericardial, dermal, vein, and synthetic grafts for tunica albuginea substitution using a rat model. *BJU Int*, **92**: 119, 2003
- Hern, W. N.: Correlation of fetal age and measurements between 10 and 26 weeks of gestation. *Obstet Gynecol*, **63**: 26, 1984
- Cardoso, L. E. and Mourao, P. A.: Glycosaminoglycan fractions from human arteries presenting diverse susceptibilities to atherosclerosis have different binding affinities to plasma LDL. *Arterioscler Thromb*, **14**: 115, 1994
- Cabral, C. A. P., Sampaio, F. J. B. and Cardoso, L. E. M.: Analysis of the modifications in the composition of bladder glycosaminoglycan and collagen as a consequence of changes in sex hormones associates with puberty or oophorectomy in female rats. *J Urol*, **170**: 2512, 2003
- Kim, K. M., Kogan, B. A., Massad, C. A. and Huang, Y.-C.: Collagen and elastin in the normal fetal bladder. *J Urol*, **146**: 524, 1991
- Shen, Z. J., Jin, X. D., Chen, Z. D. and Shi, Y. H.: Effect of aging on penile ultrastructure. *Asian J Androl*, **3**: 281, 2001

17. Da-Silva, E. A., Sampaio, F. J. B., Dornas, M. C., Damião, R. and Cardoso, L. E. M.: Extracellular matrix changes in urethral stricture disease. *J Urol*, **168**: 805, 2002
18. Hiltunen, E. L., Anttila, M., Kultti, A., Ropponen, K., Penttinen, J., Yliskoski, M. et al: Elevated hyaluronan concentration without hyaluronidase activation in malignant epithelial ovarian tumors. *Cancer Res*, **62**: 6410, 2002
19. Siroky, M. B. and Azadzi, K. M.: Vasculogenic erectile dysfunction: newer therapeutic strategies. *J Urol*, **170**: S24, 2003
20. Weinberg, P. D., Carney, S. L., Winlove, C. P. and Parker, K. H.: The contributions of glycosaminoglycans, collagen and other interstitial components to the hydraulic resistivity of porcine aortic wall. *Connect Tissue Res*, **36**: 297, 1997