Stereological analysis of smooth muscle cells and collagen fibers in experimental diabetic rabbit penis

Figueiredo, MA.[1], Ribeiro, ICA.[1], Chagas, MA.[2], Babinski, MA.[2], Maia, RS.[3], Costa, WS.[3] and Sampaio, FJB.[3]

[1]Universidade Federal Rural do Rio de Janeiro
[2]Universidade Federal Fluminense
[3]Universidade Estado do Rio de Janeiro

The general understanding of the morphological changes and physiology of penile erection is obtained through several studies considering different animal models. The aim of this study is to verify the effect of the diabetes mellitus in the rabbit penis. The ethical committee of the State University of Rio de Janeiro approved the research protocol. A total of 20 adult male white New Zealand rabbits were used. Alloxan treatment was initiated at ages 8 weeks in 10 rabbits by the intravenous injection of 100 mg.kg^{-1}. alloxan monohydrate (Sigma Chemical Co., St. Louis, Missouri). A total of 10 rabbits served as the normal control group. Serum glucose levels were monitored 24, 48 and 72 hours after injection as well as each week after alloxan treatment. After 8 weeks, the euthanasia was made by an endovenous overdose of sodium thiopenthal. The penis were removed and immediately fixed in 4% phosphate buffered formalin solution and/or Bouin's liquid for 24-48 hours. Afterwards, penile mid shaft segments were processed 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 um sections were stained with Massom Trichrome to detect smooth muscle cells and collagen fibers. The data were expressed as volumetric densities (%). The analyzed fields were digitized with 400x 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. Statistical analysis The data were analyzed in the software Graphpad Instat (Graphpad). To compare the quantitative data of corpus spongiosum and corpus cavernosum in both groups the Student's t-test was used ($p < 0.05$ was considered significant). The histochemical analysis confirmed the presence of smooth muscle cells and collagen fibers in corpus cavernosum, and spongiosum in both groups. In control groups the volumetric density (vv) was: CC (49,28% of smooth muscle and 25.80% of collagen fibers) and CS(50.62% of smooth muscle and 32.02 of collagen fibers). In diabetic group the results were: CC (64.52% of smooth muscle and 14.53% of collagen fibers) and CS(34.02% of smooth muscle and 54.85 of collagen fibers). There was statistical difference in the values of corpus spongiosum and cavernosum in the amount of smooth muscle cells and collagen fibers. The present data should therefore provide important variables for devising experiments and interpreting results when using rabbit penis as a model for penile dysfunctions.