Structural Study of Prepuce in Hypospadias—Does Topical Treatment With Testosterone Produce Alterations in Prepuce Vascularization?

André Netto Bastos, Leonardo Resende Souza Oliveira, Carlos Eduardo Prata Fernandes Ferrarez, André Avarese de Figueiredo, Luciano Alves Favorito and Jose Murillo Bastos Netto*

From the Federal University of Juiz de Fora and Urogenital Research Unit, Rio de Janeiro State University, Rio de Janeiro, Brazil

Abbreviation and Acronym

vWF = von Willebrand's factor

Study received institutional review committee and parental approval.

* Correspondence: Av. Rio Branco, 2985/sl. 605, Juiz de Fora-MG, Brazil, 36010012 (telephone: 55(32) 3218-9899; FAX: 55(32)3218-9899; e-mail: jmbnetto@uropedjf.com.br).

Purpose: Androgen stimulation before hypospadias surgery has resulted in increased penile size, fewer complications and improved cosmesis, and suggests increased neovascularization. To our knowledge the real effect on neovascularization remains to be proved. We studied the histological effects of testosterone on neovascularization.

Materials and Methods: A total of 26 boys with hypospadias were randomly allocated to 2 groups before surgical correction. Group 1 did not receive any treatment and group 2 received 1% testosterone propionate ointment twice daily for 30 days before surgery. During the surgical procedure a fragment of prepuce was excised and prepared for histological evaluation. The number and volume density of blood vessels were determined by labeling for von Willebrand's factor. Blood vessel quantification as volume density was done using a video microscopy system with a superimposed cycloid arch test system.

Results: The groups were similar in age and hypospadias classification. Testosterone treated prepuces (group 2) had an increased absolute number of blood vessels (mean $\pm$ SD 8.5 $\pm$ 1.3 vs 4.8 $\pm$ 1.8 vessels per field) and increased blood vessel volume density (mean 50.5% $\pm$ 7.8% vs 24.8% $\pm$ 8.6% vessels per point) (each $p < 0.001$) compared to those in untreated patients (group 1).

Conclusions: The use of 1% testosterone propionate ointment before hypospadias surgery produces neovascularization in absolute numbers and in volume density.

Key Words: urethra; neovascularization, physiologic; hypospadias; testosterone; foreskin

Hypospadias is a common congenital defect and surgical correction, even in experienced hands, is prone to complications. A cosmetically adequate surgical outcome is often not attained with 25% of patients reporting dissatisfaction with the cosmetic appearance of the penis.[1] Androgen stimulation with testosterone, dihydrotestosterone or human chorionic gonadotropin has been used to improve the cosmesis and decrease the complications of hypospadias surgery since it temporarily increases the penis and makes surgical correction easier.[2] Besides a penile increase other studies have shown decreased chordee, corpus spongiosum thickening and decreased hypospadias.[3] Chalapathi et al found no differences in penile size, testosterone side effects and serum testosterone when comparing topical to intramuscular testosterone.[4]

Nevertheless, the testosterone effects that lead to better postoperative

0022-5347/11/1856-2474/0
THE JOURNAL OF UROLOGY®

Vol. 185, 2474-2478, June 2011
Printed in U.S.A.
DOI:10.1016/j.juro.2011.01.035

results are yet to be fully understood. Histology of the preoperatively androgen treated prepuce, especially targeting tissue vascularization, is paramount once greater blood flow decreases fibrous tissue formation and inflammation, decreases postoperative complications and improves cicatrization through better cutaneous nutrition and oxygenation.[5]

We analyzed the histological effects of topic testosterone applied before hypospadias surgery on preputial vascularization.

Hypospadias classification in groups 1 and 2

Hypospadias	No. Group 1	No. Group 2
Glandular	2	2
Coronal	4	5
Subcoronal	4	3
Penile:		
Mid	2	2
Posterior	1	1
Totals	13	13

MATERIALS AND METHODS

The current study received institutional review committee and parental approval. It was done in accordance with the ethical standards of the responsible institutional committee on human experimentation.

We studied untreated prepubescent children with hypospadias who did not present with any other genital or endocrine condition. After surgery the children were divided at random using the Research Randomizer (www.randomizer.org) into 2 groups. Group 1 with no testosterone included 13 children who did not receive any preoperative hormonal treatment. Group 2 with testosterone included 13 children who received 1% testosterone propionate ointment for use on the whole penis twice daily for 30 days before hypospadias surgical correction. Parents were instructed to use the same amount each time.

During the surgical procedure a small fragment of prepuce (about 1.5 cm^2) containing the inner and outer surfaces was removed. The preputial fragment was fixed in 10% buffered formalin and routinely processed for paraffin embedding with 4 μm thick sections obtained at 200 μm intervals.[6,7] The material was stained with hematoxylin and eosin for histological examination. The pathologist evaluated the preputial fragment as a spiral including 10 random fields in which the inner and outer surfaces of the skin were examined as 1 piece.

The streptavidin-biotin-peroxidase method was used for immunohistochemical staining for vWf.[8] Three randomly chosen microscopic fields at 200× magnification were photographed. The absolute number of blood vessels per field was counted and the mean number of blood vessels per field was determined. Using a microscope adapted video system, including a DMR DMRBE microscope (Leica, Exton, Pennsylvania), a Prior® video camera and a Trinitron® monitor, 3 randomly chosen 200× fields were photographed. Cycloid points were superimposed on the images. The vessels that touched the cycloid points were counted and those that did not touch were discarded (fig. 1, *A*). Vascular volume density (VV) was then determined using the formula, VV = PP/TP, where PP corresponds to the number of vessels touching each 1 of the 16 cycloid points and TP corresponds to the total number of points (16).[9,10]

The means were statistically compared using the unpaired t test with Prism® software. Results were considered statistically significant at $p < 0.05$.

RESULTS

The 2 groups were homogeneous according to mean age and hypospadias classification (see table). In group 1 mean ± SD age was 67.5 ± 48.1 months (range 4 to 120) and in group 2 it was 41.3 ± 36.5 months (range 12 to 120). Analysis of immunohistochemical staining for vWF revealed a significant increase in the absolute number of preputial blood vessels in group 2 compared to those in group 1 (mean 8.5 ± 1.3 vs 4.8 ± 1.8 vessels per field, $p < 0.001$, figs. 1, *B* and *C* and 2, *A*). Structural

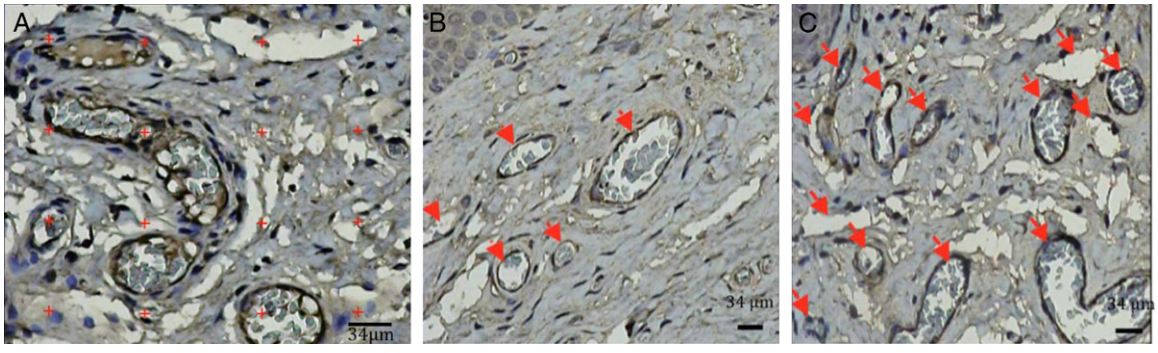

Figure 1. Photomicrographs show preputial morphometric analysis. *A*, blood vessel quantification by 16 cycloid points (plus marks) in microscopic field in group 1 patient without testosterone at 69 months. *B* and *C*, histological view of quantification of blood vessels per field (arrows) at 16 months. *B*, group 1 patients without testosterone. *C*, group 2 patient with testosterone. vWF, reduced from ×200.