Structural Study of Gubernaculum Testis in Fetuses with Prune Belly Syndrome

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Abbreviations and Acronyms

CGRP = calcitonin gene-related peptide

PBS = prune belly syndrome

Vv = volumetric density

WPC = weeks of gestation

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Purpose: We compared and contrasted the structure of the gubernaculum testis in fetuses with prune belly syndrome and normal controls.

Materials and Methods: We studied a total of 6 gubernacula from 3 male fetuses with prune belly syndrome and a total of 14 from 7 male fetuses without an anomaly. Gubernacular specimens were cut into 5 μm sections and stained with Masson trichrome to quantify connective tissue and smooth muscle cells, with Weigert stain to observe elastic fibers and with picrosirius red with polarization to observe collagen. Immunohistochemical analysis was done with tubulin to observe the nerves. Images were captured with a BX51 microscope and DP70 camera (Olympus®). Stereological analysis was done with Image-Pro and ImageJ (MediaCybernetics®) using a grid to determine volumetric density. Means were statistically compared with the Mann-Whitney test. All tests were 2-sided with $p < 0.05$ considered statistically significant.

Results: Prune belly syndrome fetuses were at 17 to 31 weeks of gestation and control fetuses were at 12 to 35 weeks of gestation. Quantitative analysis showed no difference in the volumetric density of smooth muscle cells in prune belly syndrome vs control gubernacula (mean 15.70% vs 19%, $p = 0.2321$). Collagen fiber analysis revealed a predominance of green areas in prune belly syndrome gubernacula, suggesting collagen type III, and a predominance of red areas in control gubernacula, suggesting collagen type I. Elastic fibers were significantly smaller in prune belly syndrome gubernacula than in control gubernacula (mean 14.06% vs 24.6%, $p = 0.0190$). Quantitative analysis demonstrated no difference in the volumetric density of nerves in prune belly syndrome or control gubernacula (mean 5.200% vs 3.158%, $p = 0.2302$).

Conclusions: The gubernaculum in fetuses with prune belly syndrome had altered concentrations of collagen and elastic fibers. These structural alterations could be one of the factors involved in cryptorchidism in prune belly syndrome.

Key Words: testis, prune belly syndrome, cryptorchidism, collagen, elastic tissue

Prune belly syndrome is a disorder characterized by abdominal muscle deficiency or hypoplasia, urinary tract malformation such as a large, hypotonic bladder and dilated, tortuous ureters, and bilateral cryptorchidism.[1] Urethral obstruction is present in a third of patients with PBS, which could be the primary cause of the malformations in this syndrome.[2,3] To our knowledge the cause of cryptorchism in this syndrome is unknown.

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However, it is speculated that anatomical changes in the anterior abdominal wall hinder an increase in intra-abdominal pressure, which is one of the factors needed for testicular descent. It was also speculated that the large bladder in this syndrome makes the inguinal canal extraperitoneal so that the gubernaculum and its contained processus vaginalis cannot develop normally in the inguinal canal.[2,3]

Various factors have been proposed as the causative agent of testicular descent in humans, including increased intra-abdominal pressure,[4,5] development of the epididymis, spermatic vasa, deferential ducts and inguinal canal,[6] stimuli from the genitofemoral nerve,[7] hormonal stimulus originating in placental gonadotrophin and testosterone produced by the fetal testes,[8] and gubernacular development.[6]

The gubernaculum seems to be the most important anatomical structure in the process of testicular descent. The gubernaculum is an elongated, cylindrical structure that connects the inferior pole of the testis and the tail of the epididymis to the inguinal canal and scrotum.[6,9] It is composed of an abundant and often loose extracellular matrix and mesenchymal cells such as fibroblasts and smooth muscle cells.[10] The role of the gubernaculum during testicular descent has been explained mainly by its capacity for dilatation and contraction.[6,10]

Previous groups assessed gubernacular structure in human fetuses and in patients with cryptorchidism.[9–12] However, to our knowledge there is no morphological study in the literature of the gubernaculum testis in patients or fetuses with PBS. We hypothesized whether the structure of the gubernaculum in fetuses with PBS is similar to that in normal fetuses and whether increased intra-abdominal pressure in PBS causes alterations in gubernacular structure. Thus, we compared and contrasted the structure of the gubernaculum testis in fetuses with PBS and in normal controls.

MATERIALS AND METHODS

The experimental protocol was approved by the ethical committee for human experimentation at our university. This study was performed in accordance with the ethical standards of the hospital institutional committee on human experimentation.

We studied 6 gubernacula from a total of 3 male fetuses with PBS and 14 from a total of 7 male fetuses without an anomaly. The fetuses were macroscopically well preserved. Fetal gestational age was determined in WPC according to the foot length criterion, which is currently considered the most acceptable parameter to calculate gestational age.[13–15] Fetal crown-rump length and body weight were also evaluated immediately before dissection. The same observer analyzed the measurements.

After measurements the fetuses were dissected using a stereoscopic lens at 16/25× magnification. The abdomen and pelvis were opened to identify and expose the urogenital organs and inguinal canal, and reveal testicular position.

Testicular position was classified after dissection as 1) abdominal when the testis was proximal to the internal ring, 2) inguinal when the testis was found between the internal and external inguinal rings, and 3) scrotal when the testis had passed beyond the external inguinal ring and was in the scrotum. We observed proximal and distal insertions of the gubernaculum and the structure of the inguinal canal (fig. 1). The relationship between the testis and the epididymis was also evaluated.

The testis and gubernaculum were separated from the other structures, fixed in 10% buffered formalin and routinely processed for paraffin embedding. Sections (5 μm) were obtained at 200 μm intervals. Smooth muscle and connective tissue, elastic system fibers and collagen were studied by histochemical and immunohistochemical methods.

Sections were stained with hematoxylin and eosin to assess tissue integrity. Other staining methods were also used, including Masson trichrome to quantify connective and smooth muscle tissue, Weigert resorcin fucsin with previous oxidation to observe elastic system fibers and picrosirius red with polarization to observe different collagen types. Gubernacular nerves were analyzed immunohistochemically with β III tubulin (mouse monoclonal antibody).

Connective and smooth muscle tissues, nerves and elastic system fibers were quantified by a stereological method.[16–18] For quantitative analysis we studied 5 microscopic fields chosen at random for a total of 25 test areas per gubernaculum. We used ImageJ, version 1.46r, loaded with a plug-in (http://rsb.info.nih.gov/ij/). All sections were photographed with a DP70

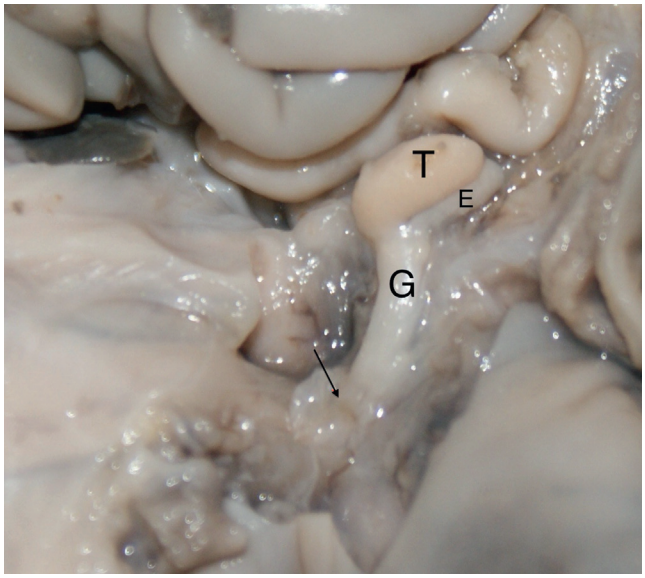

Figure 1. Control fetus at 21 WPC. Anterior abdominal wall was extirpated and each testis was in abdomen. Note relationship between left testis (*T*), epididymis (*E*) and gubernaculum (*G*). Arrow indicates internal inguinal ring.