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TESTICULAR MIGRATION: REMODELING OF CONNECTIVE TISSUE AND MUSCLE CELLS IN HUMAN GUBERNACULUM TESTIS

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ABSTRACT

Purpose: We present the main morphological modifications in the human gubernaculum during testicular migration in humans.

Materials and Methods: We obtained 12 gubernacula from fresh, macroscopically normal human fetuses at 15 to 29 weeks of gestation. Collagen was evidenced using trichrome and Sirius red staining procedures, while Weigert's resorcinol-fuchsin and anti-human elastin antibody were used to reveal elastic system fibers. Smooth muscle cells were detected by anti-human smooth muscle α-actin antibody.

Results: When the testes were still located in the abdomen at 15 to 16 weeks of gestation, collagen fibers were sparse and embedded in a loose extracellular matrix. The amount of fibers then gradually increased with age and at 28 weeks of gestation the gubernaculum was mostly collagenous in composition. Elastic fibers had a similar growth pattern, although they were located mainly at the distal end of the gubernaculum. Fibroblasts largely predominated over other cell types and decreased in number with gestational age, whereas smooth muscle cells were restricted to the walls of blood vessels. Striated muscle cells were detected at the scrotal end of the gubernaculum, where they were disposed as isolated and scattered bundles running in various directions. Like fibroblasts, their number also decreased with age.

Conclusions: During testicular migration gubernacular connective tissue undergoes extensive remodeling and ultimately becomes an essentially fibrous structure rich in collagen and elastic fibers. Such changes should decrease the size of the gubernaculum and, thus, contribute to other forces that cause the testes to move toward the scrotum. In fact, because of the lack of smooth muscle cells, and the amount and organization of striated muscle cells, active contraction of the gubernaculum is less likely to be an important factor in testicular descent.

KEY WORDS: testis, fetus, scrotum, connective tissue, cryptorchidism

In human fetuses the testes normally migrate from the abdomen to the scrotum, traversing the abdominal wall between the 15th and 28th weeks of gestation (17th to 30th menstrual weeks). Complications that adversely affect this displacement may lead to cryptorchidism and other testicular abnormalities. However, the mechanisms that regulate testicular migration are not yet well established. The most accepted theories for explaining testicular descent in humans are related to increased intra-abdominal pressure,[1,2] the development of the epididymis, vas spermatic, vas deferens and inguinal canal,[1] development of the gubernaculum,[3,4] stimulus from the genitofemoral nerve[4] and various stimuli from hormones and biologically active peptides with systemic and/or paracrine effects.[2,5–7]

The gubernaculum is an elongated and cylindrical mesenchymal structure that connects the inferior pole of the testis and the tail of epididymis to the scrotum.[3,4] Histologically the gubernaculum is composed of an abundant extracellular matrix that is rich in glycosaminoglycans (GAG)s and mesenchymal cells, such as fibroblasts and smooth muscle cells.[3,4] Putative morphological and compositional changes in the gubernaculum are considered to have an important role in testicular migration. In fact, the gubernaculum undergoes extensive remodeling during this process[3] and yet the organization and composition of its extracellular matrix and cells remain poorly known, especially in human tissue.

The role of the gubernaculum during testicular migration has been explained mainly by its capacity of dilatation and contraction.[3,4] The gubernaculum increases in volume mainly during the second trimester of gestation, when the testis passes through the inguinal canal, most likely due to increased GAG synthesis.[4] It is supposed that the increase in gubernaculum volume is important for facilitating testicular passage through the inguinal canal.[3–5] Although the mechanisms that regulate these marked structural modifications are still not well known, experimental evidence suggests that several and diverse hormone or other soluble factor may be involved,[2] including the nonandrogen hormone insulin-like factor-3,[8,9] androgens that may act in paracrin fashion[10] and calcitonin gene-related peptide, which is a neuromuscular transmitter.[2]

Experimental studies in various animal models show modifications in the gubernaculum during testicular migration, demonstrating that this structure is of the utmost importance for this process.[11–15] Some studies of morphological macroscopic aspects of the testicular migration have been done in human fetuses.[16–18] Nevertheless, to our knowledge a detailed analysis of the molecular and cellular components of gubernacular connective tissue is lacking. We correlated the morphology of the gubernaculum with testicular position in human fetuses at different gestational ages, focusing analysis on its principal components, that is elastic fibers, muscle cells and collagen.

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The position of the testis and fetal parameters

No. Gestational Wks.	Wt. (gm.)	Crown/Rump Length (cm.)	Rt. Testis	Lt. Testis
15	170	15	Abdominal	Abdominal
15	225	17	Abdominal	Abdominal
16	190	14	Abdominal	Abdominal
20	381	18	Abdominal	Abdominal
20	468	18	Abdominal	Abdominal
20	371	19	Abdominal	Abdominal
24	865	25	Canalicular	Canalicular
24	660	20	Abdominal	Abdominal
25	698	23	Abdominal	Abdominal
28	1,415	36.5	Scrotal	Scrotal
28	1,291	27.5	Scrotal	Scrotal
29	1,190	27	Scrotal	Scrotal

We studied 12 right gubernacula obtained from 12 fresh human fetuses that died of causes unrelated to the urogenital tract. The fetuses were macroscopically well preserved and no signs of congenital malformation were detected. The gestational age of 15 to 29 weeks, corresponding to 17 to 31 menstrual weeks, was estimated according to foot length criteria.[19–22] The fetuses were also evaluated in regard to crown-rump length and body weight immediately before dissection (see table).

After fetal classification the abdomen, inguinal canal and scrotum were opened to identify and remove the testes, epididymides and gubernacula. The removed tissue samples were immediately immersed in Bouin fixative for 48 to 72 hours. The material was then processed by routine histological methods and embedded in paraffin, from which 5 μm. sections were obtained.

For histochemical analysis collagen fibers were stained with Gomori's and Masson's trichrome as well as with Sirius red.[23] The different forms of elastic fibers, comprising the so-called elastic fiber system, were visualized by Weigert's resorcinol-fuchsin technique with and without previous oxidation by peracetic acid.[23] For immunolabeling the standard avidin-biotin conjugate immunostaining procedure with appropriate positive and negative controls was used. To detect elastic fibers we used monoclonal antibody No. 25011/165 (Institut Pasteur, Lion, France) against human elastin, diluted 1:2. To detect smooth muscle cells we used A-2547 anti-human smooth muscle α-actin monoclonal antibody (Sigma Chemical Co., St. Louis, Missouri) according to previously described techniques.[24–26]

RESULTS

Cellular components. Fibroblasts were the most abundant cell type in the gubernaculum during the gestational period studied. Identification of these cells was based on their location shape (figs. 1 and 2, *A*) and because they failed to stain with anti-human smooth muscle α-actin antibody (fig. 3). Early in gestation when the testes were abdominal in position, fibroblasts were homogeneously distributed in the whole gubernaculum at relatively high density (figs. 1, *A* and *B*, and 2, *A*). Cell density then decreased sharply with age, so that by 29 weeks of gestation when the testes were in the scrotum, the intercellular space was noticeably larger (fig. 4). Smooth muscle cells were absent from the gubernacular tissue between weeks 15 and 29, as revealed by immunostaining sections with anti-smooth muscle α-actin antibody. Accordingly positive antibody staining was observed only in blood vessel walls (fig. 3).

Elongated cells organized as discrete bundles were the other major cell type present in the gubernaculum (figs. 1, *A*, 4 and 2, *A* and *B*). Higher magnification photomicrographs showed a characteristic cytoplasmic banding pattern (fig. 2, *B*), thereby indicating that they were striated muscle cells. Throughout the gestational period studied the muscle cell bundles were preferentially located at the distal end of the gubernaculum and their amount decreased with age. In samples from 29-week fetuses there were fewer muscle bundles, which were replaced by fibrous connective tissue (fig. 4). Notably the bundles tended to be disposed transversely in relation to the long axis of the gubernaculum (fig. 1, *A*).

Collagen. By 15 weeks of gestation when the testes were located in the abdomen, the gubernaculum had a rather loose and hyaline extracellular matrix (figs. 1, *B* and 2, *A*) with few collagen fibers, as indicated by Gomori's trichrome staining (fig. 2, *A*). In fetal samples from weeks 20 to 24 the amount of collagen increased and, thus, the extracellular matrix became moderately dense (figs. 5 and 2, *A*). At 28 weeks Sirius red collagen (fig. 2, *C*) and Masson's trichrome (fig. 2, *B*) stains revealed that the extracellular matrix was already dense with abundant collagen fibers. At 29 weeks the extracellular matrix was even denser and the gubernacular tissue was reminiscent of mature scar (fig. 4).

Elastic fibers. The expression of elastic fibers in the gubernaculum from weeks 15 to 29 was similar to that of

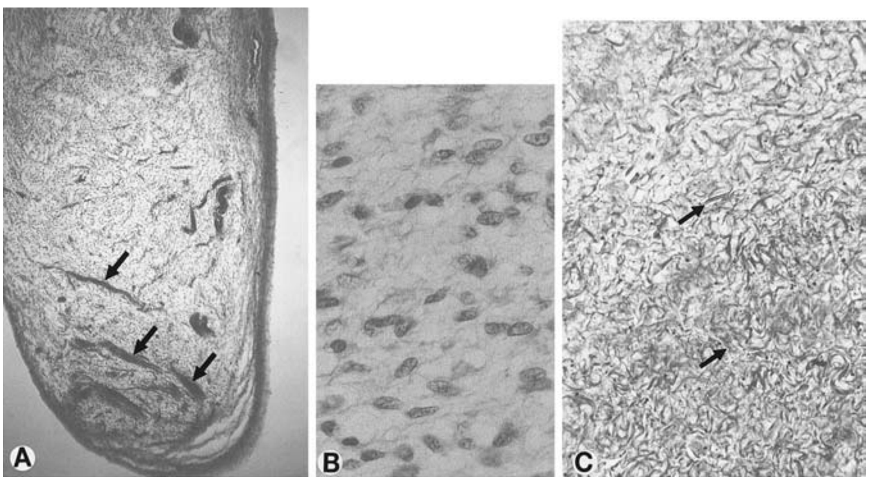

FIG. 1. Cellular components in human gubernaculum during fetal development. *A*, longitudinal section of gubernaculum from fetus 20 weeks after conception reveals that collagen fibers form a dense sheath around gubernaculum but elsewhere density is low and extracellular matrix is mostly hyaline. Bundles of muscle cells (arrows) localize at distal end, yet are absent from other regions of gubernaculum. Note that bundles are mainly oriented transversely with regard to long axis of gubernaculum. Gomori's trichrome, reduced from ×40. *B*, fibroblast-like cells with typical elongated nuclei at distal end of gubernaculum from fetus 15 weeks after conception. Note uniformity of cell population. H&E, reduced from ×400. *C*, section from fetus 28 weeks after conception reveals dense organization of collagen fibers and predominance of sparsely distributed fibroblast-like cells (arrows). Masson's trichrome, reduced from ×400.

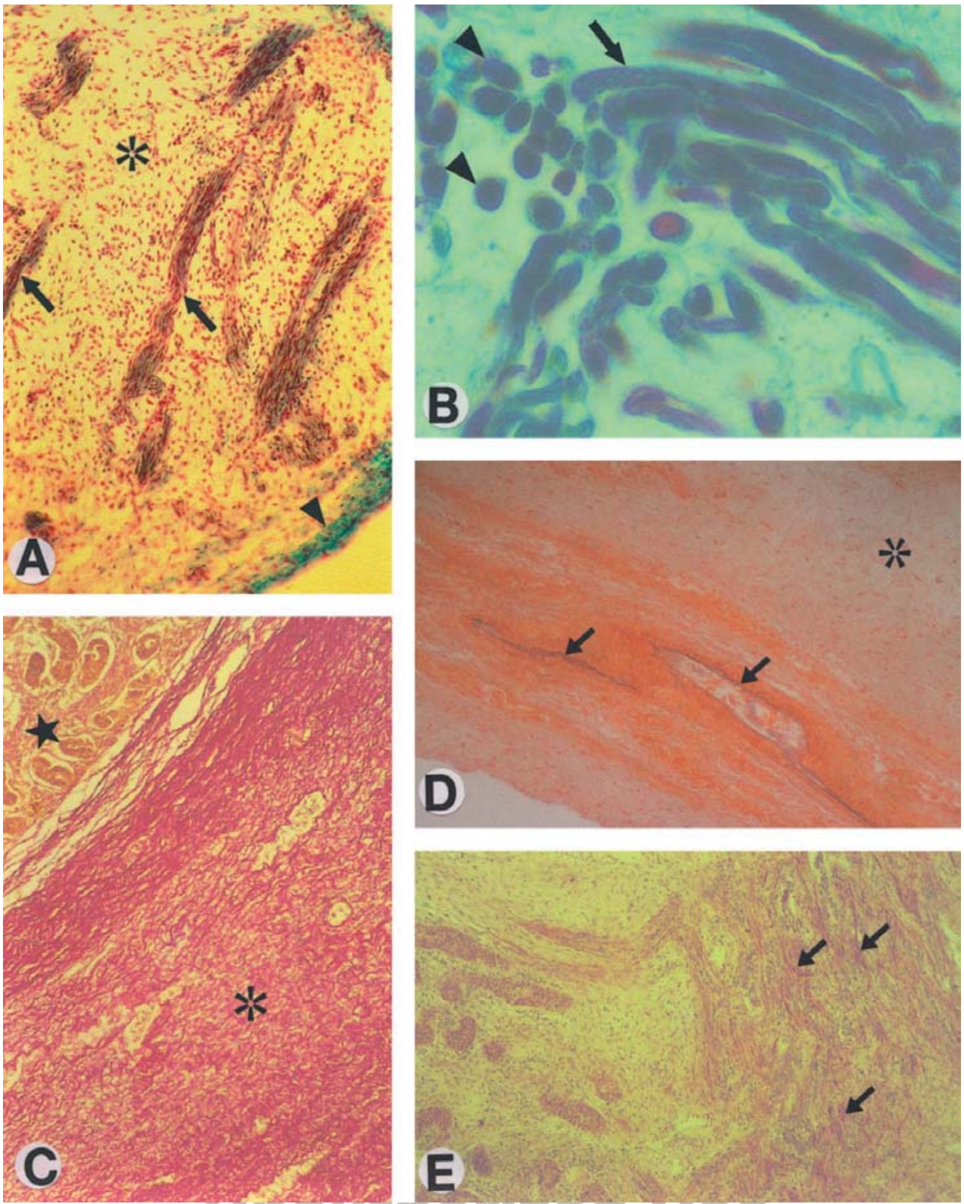

FIG. 2. Cellular components, collagen and elastic system fibers in human gubernaculum during fetal development in humans. *A*, distal end of gubernaculum from fetus 15 weeks after conception. Gubernaculum is largely composed of loose, hyaline extracellular matrix (asterisk), whereas fibrous, collagenous components are mostly restricted to periphery (arrowhead). Embedded in extracellular matrix are numerous, evenly distributed fibroblast-like cells and few bundles of muscle cells (arrows). Gomori's trichrome, reduced from ×100. *B*, higher magnification of muscle cell bundles in distal end of gubernaculum show transversely (arrowheads) and longitudinally sectioned (arrow) cells. In latter cells banding pattern is clearly noticeable. Note also presence of collagen fibers (stained blue-green) throughout interstice. Sample from fetus 24 weeks after conception. Gomori's trichrome, reduced from ×1,000. *C*, sample from fetus 28 weeks after conception shows dense collagen matrix (asterisk) at proximal end of gubernaculum where it attaches to testis (star). Sirius red, reduced from ×40. *D*, periphery of distal portion of gubernaculum from fetus 20 weeks after conception. Section was treated with Weigert's resorcinol-fuchsin with previous oxidation, and no significant amount of elastic fibers was detected in gubernacular tissue (asterisk). However, tunica intima of blood vessels (arrows) was positively stained, which confirms specificity of histochemical technique. Reduced from ×100. *E*, elastic fibers (arrows) at distal end of gubernaculum in close association with muscle cell bundles. Sample from fetus 28 weeks after conception. Anti-human elastin antibody, reduced from ×100.

collagen (figs. 6 and 2, *D* and *E*). Thus, in fetal samples from weeks 15 to 20 no elastic fibers were detected by anti-human elastin antibody (data not shown) or by Weigert's resorcinol-fuchsin (figs. 5, *A* and 2, *D*). On the other hand, in the same sections the internal elastic membrane of blood vessels had a clearly positive reaction, confirming absent elastic fibers in the gubernacular tissue proper (fig. 2, *D*). By week 28 each staining technique revealed conspicuous elastic fibers, (fig. 5, *B* and *C*) preferentially located at the distal end of the gubernaculum (fig. 2, *E*). At this part of the gubernaculum elastic fibers gradually replaced striated muscle cell bundles (fig. 2, *E*).

DISCUSSION

Previous studies of the histological organization of the gubernaculum in human fetuses were usually quite limited in scope, describing this structure as containing abundant extracellular matrix and a large number of undifferentiated cells.[3–5,8] The gubernaculum undergoes extensive remodeling during the fetal and postnatal periods, and a number of experiments in animals variously demonstrated that this structure is implicated in testicular migration.[27–30] However, experimental animals may not be good models for testicular migration in humans, a notion that was already mentioned

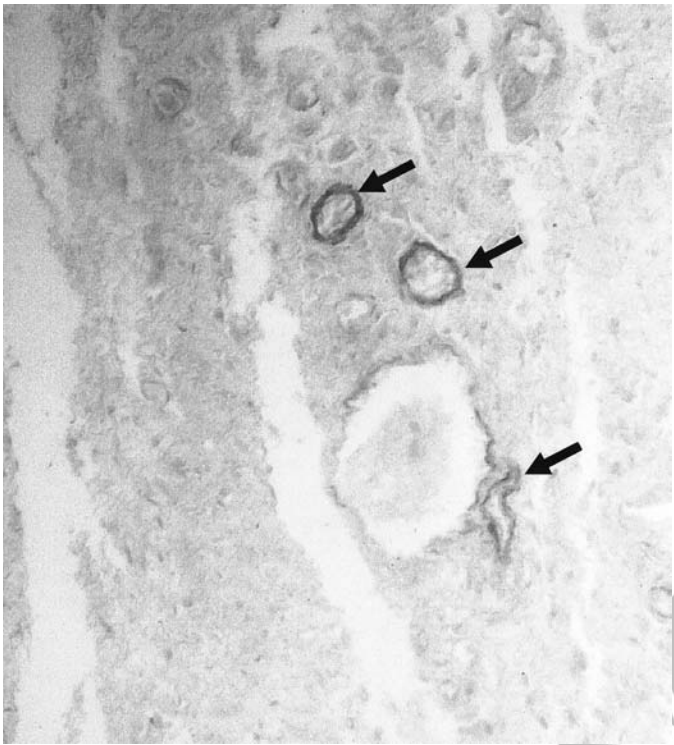

FIG. 3. Immunostaining of gubernaculum with antismooth muscle α-actin antibody. Positivity to antibody shows that smooth muscle cells in gubernaculum are restricted to wall of blood vessels (arrows). Sample from fetus at 28 weeks after conception. Reduced from ×100.

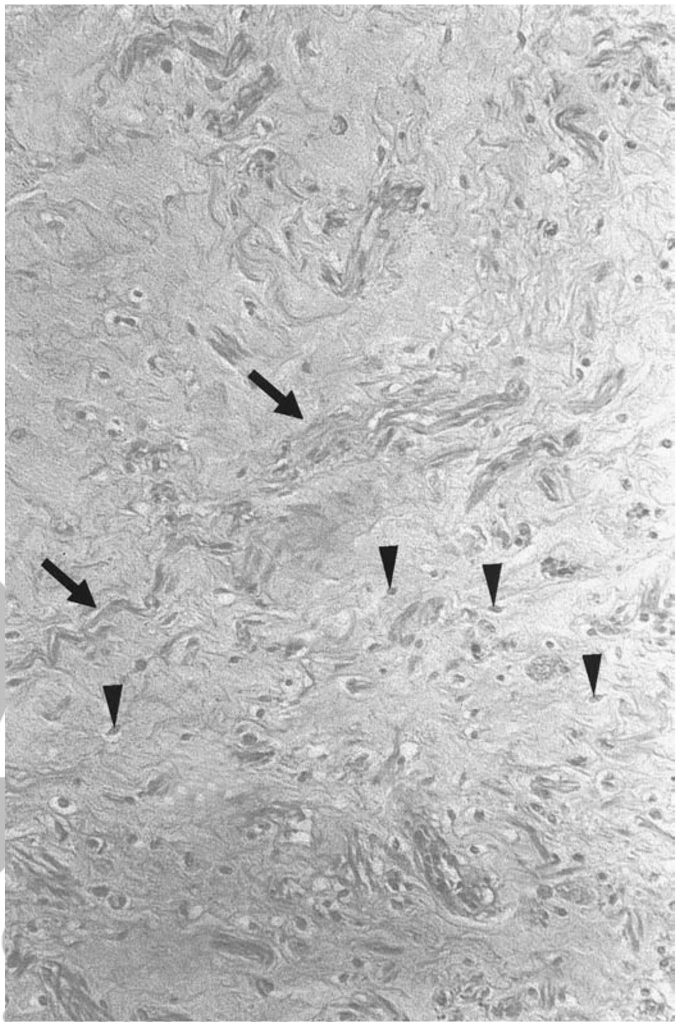

FIG. 4. At 29 weeks after conception gubernaculum is essentially fibrous structure composed of densely packed collagen fibers interspersed with widely separated fibroblast-like cells (arrowheads) and few striated muscle cell bundles (arrows). Distal end of gubernaculum. H&E, reduced from ×100.

20 years ago.[5] In addition to important anatomical differences,[28] descent in such animals usually occurs long after birth and, hence, under different physiological conditions and regulatory mechanisms.

Our results show that in the early phases of testicular migration the gubernaculum is a hydrated structure with a loose extracellular matrix and numerous fibroblasts. As the testes move toward the scrotum, it becomes progressively fibrous with low cellularity. This finding correlates with the data of Heyns, who reported that the wet-to-dry mass ratio of the gubernaculum decreases after testicular descent.[3] A decrease in the concentration of GAGs, major extracellular matrix molecules capable of retaining water, also occurs in the gubernaculum during testicular migration in pigs.[14] This rapid remodeling of gubernacular connective tissue, which was noted in our samples at 20 to 24 weeks of gestation also confirms our previous anatomical observations that testicular passage in the inguinal canal at this time in gestation[16,18] is likewise rapid.[5]

In our samples elastic fibers were concentrated at the distal end of the gubernaculum and appeared only later in testicular descent. To our knowledge we report the first description of elastic fibers in the human gubernaculum. It is common knowledge that after birth and in individuals with normally descended testes the gubernaculum differentiates into fasciae associated mainly with the testis and epididymis.[5] Due to our results we hypothesize that these elastic fibers in an already fibrous gubernaculum are actually the initial stages in the formation of a greater amount of fibers for those fasciae. In addition, our finding of a discrete collagenous capsule around the gubernaculum supports the view that it usually lies loose in the mesenchyma of the inguinoscrotal region.[4] This fact would enable the tip of the gubernaculum to be motile. The bundles of striated muscle at the distal end of the gubernaculum may be the attachment fibers of the cremaster muscle, which adheres to the distal part of the gubernaculum.[2,4,5] However, in this study we did not identify morphological evidence relating these bundles to the cremaster muscle.

Another important feature revealed by our morphological analysis was the overall paucity of muscular cells in the gubernaculum during the fetal period when testicular descent occurs. Based on smooth muscle α-actin staining we can infer that smooth muscle cells are completely lacking and myofibroblasts should not be present in significant numbers since the phenotypic modulation of fibroblasts into cells is usually accompanied by the expression of large amounts of α-actin.[31] In addition, striated muscle cells were sparse and transversely oriented in regard to the gubernacular long axis. These findings suggest that unlike in rats[15,29] the human gubernaculum should not be capable of significant contraction. These observations also imply that if the gubernaculum pulls the testis down from the abdomen into the scrotum in humans, it should be much more a result of a remodeling and consequent shortening of its connective tissue rather than of an active, muscle mediated contraction. Another corollary of the noncontractility of the human gubernaculum is that it provides further support to the proposition of Husmann and Levy concerning calcitonin gene-related peptide.[2] They argued that this factor should not have a significant role in testicular migration in humans since it is essentially a neuromuscular transmitter. The transverse fibers of striated muscle at the distal tip of the

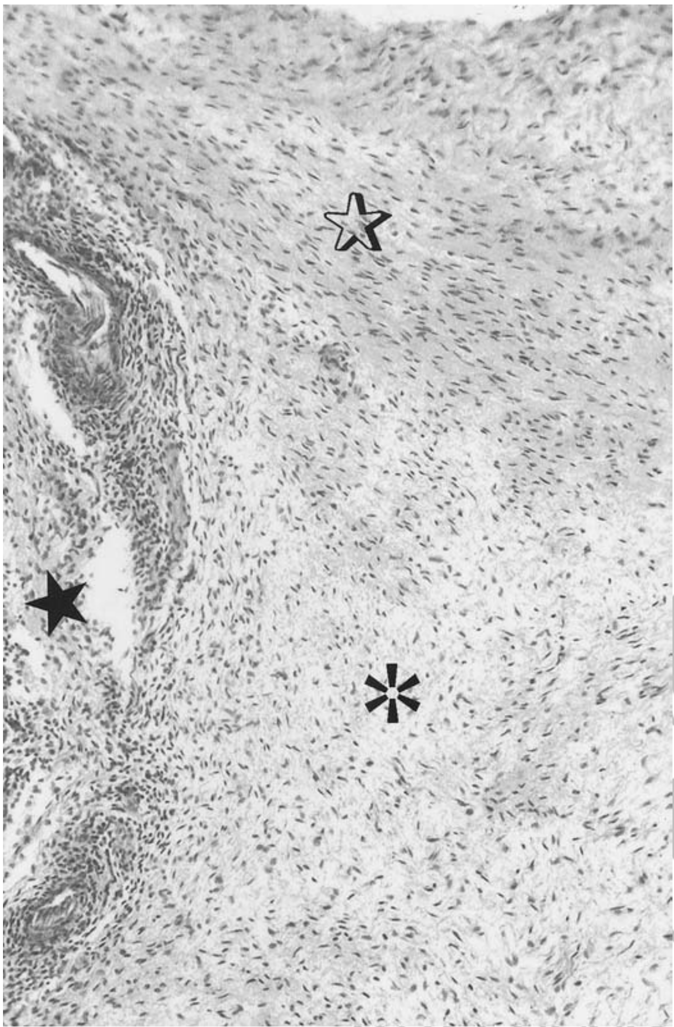

FIG. 5. Proximal end of gubernaculum shows its close attachment to testis (closed star). Gubernaculum presents areas of denser (open star) and looser (asterisk) connective tissue. Sample from fetus 20 weeks after conception. H&E, reduced from ×40.

human gubernaculum may serve a function similar to that in rats, in which they orient testicular direction.[15,29]

Current experimental evidence indicates that the dramatic changes undergone by gubernacular tissue during testicular descent, as shown by our results, are mediated by a complex and as yet unclear array of stimulatory and inhibitory factors. Accordingly the testicular paracrine factor descendin is thought to cause in an androgen independent manner a swelling of the gubernaculum, thereby, facilitating the passage of the testes through the inguinal canal.[32] This swelling corresponds to the loose matrix that we observed in samples by 15 weeks of gestation. On the other hand, estrogens would inhibit this effect, directly or by down regulating the expression of insulin-like growth factor-3,[8] which is an enhancer of gubernacular growth.[9,33] Interestingly while it was reported that the regression or shortening of the gubernaculum depends on androgens,[10] others noted that these hormones may also stimulate gubernacular outgrowth.[9] Notably many of these results were obtained in experimental animals. Thus, as mentioned, the implied physiological mechanisms should be applied to humans with caution.

Although the extent of this shortening of the gubernaculum and the traction force it would exert cannot currently be ascertained, as discussed it should act synergistically with other factors that are thought to cause the testis to migrate from abdomen to scrotum. In this context cryptorchidism should be viewed as a pathological condition with multiple etiologies.

CONCLUSIONS

During the period in which testicular migration occurs gubernacular connective tissue undergoes extensive remodeling and ultimately becomes an essentially fibrous structure rich in collagen and elastic fibers. Such changes should decrease the size of the gubernaculum and, thus, contribute to other forces that cause the testes to move toward the scrotum. In fact, due to the organization and small amount of muscular cells active contraction of the gubernaculum is less likely to be an important factor in testicular descent in humans.

REFERENCES

1. Hadziselimovic, F.: Mechanism of testicular descent. Urol Res, **12:** 155, 1984

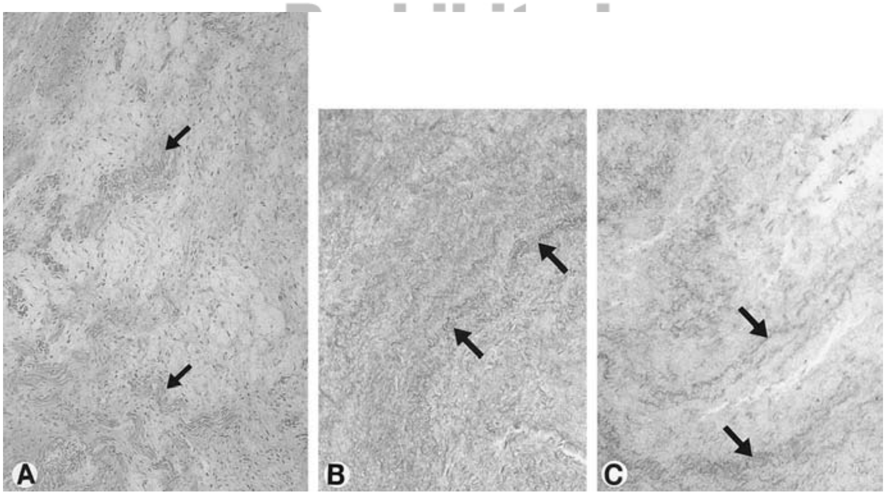

FIG. 6. Elastic system in human gubernaculum during fetal development. *A*, distal portion of gubernaculum from fetus 15 weeks after conception stained with Weigert's resorcinol-fuchsin after previous oxidation of sections. Histochemical technique is capable of revealing different forms of elastic fibers, none of which is present in section. Arrows show bundles of muscle cells. Reduced from ×100. *B*, abundant bundles of elastic fibers (arrows) in distal end of gubernaculum from fetus 28 weeks after conception. Anti-human elastin antibody, reduced from ×100. *C*, distal portion of gubernaculum from fetus 28 weeks after conception. Note that amount and organization of elastic fibers (arrows) are nearly identical to those shown in part *B*. Weigert's resorcinol-fuchsin stain, reduced from ×100.

2. Husmann, D. A. and Levy, J. B.: Current concepts in the pathophysiology of testicular undescent. Urology, **46:** 267, 1995
3. Heyns, C. F.: The gubernaculum during testicular descent in the human fetus. J Anat, **153:** 93, 1987
4. Heyns, C. F. and Hutson, J. M.: Historical review of theories on testicular descent. J Urol, **153:** 754, 1995
5. Backhouse, K. M.: Embryology of testicular descent and maldescent. Urol Clin North Am, **9:** 315, 1982
6. Elder, J. S., Isaacs, J. T. and Walsh, P. C.: Androgenic sensitivity of the gubernaculum testis: evidence for hormonal/mechanical interactions in testicular descent. J Urol, **127:** 170, 1982
7. Rafjer, J. and Walsh, P. C.: Hormonal regulation of testicular descent: experimental and clinical observations. J Urol, **118:** 985, 1977
8. Emmen, J. M., McLuskey, A., Adham, I. M. et al: Involvement of insulin-like factor 3 (Insl3) in diethylstilbestrol-induced cryptorchidism. Endocrinology, **141:** 846, 2000
9. Emmen, J. M., McLuskey, A., Adham, I. M. et al: Hormonal control of gubernaculum development during testis descent: gubernaculum outgrowth in vitro requires both insulin-like factor and androgen. Endocrinology, **141:** 4720, 2000
10. McMahon, D. A., Kramer, S. A. and Husmann, D. A.: Antiandrogen induced cryptorchidism in the pig is associated with failed gubernacular regression and epididymal malformations. J Urol, **154:** 553, 1995
11. Heyns, C. F., Husmann, H. J. and De Klerk, D. P.: Hyperplasia and hypertrophy of the gubernaculum during testicular descent in the fetus. J Urol, **135:** 1043, 1986
12. Attah, A. A. and Hutson, J. M.: The role of intra-abdominal pressure in cryptorchidism. J Urol, **150:** 994, 1993
13. Abe, T., Aoyama, K., Gotoh, T. et al: Cranial attachment of the gubernaculum associated with undescended testes. J Pediatr Surg, **31:** 652, 1996
14. Heyns, C. F., Husmann, H. J., Werely, C. J. et al: The glycosaminglycans of the gubernaculum during testicular descent in the fetus. J Urol, **143:** 612, 1990
15. Park, W. H. and Hutson, J. M.: The gubernaculum shows rhythmic contractility and active movement during testicular descent. J Pediatr Surg, **26:** 615, 1991
16. Sampaio, F. J. B. and Favorito, L. A.: Analysis of testicular migration during the fetal period in humans. J Urol, **159:** 540, 1998
17. Favorito, L. A. and Sampaio, F. J. B.: Anatomical relationships between testis and epididymis during the fetal period in humans (10–36 weeks postconception). Eur Urol, **33:** 121, 1998
18. Favorito, L. A., Sampaio, F. J. B., Javaroni, V. et al: Proximal insertions of gubernaculum testis in normal human fetuses and in boys with cryptorchidism. J Urol, **164:** 792, 2000
19. Streeter, G. L.: Weight, sitting height, head size, foot length and menstrual age of the human embryo. Contr Embryol Carnegie Inst, **11:** 143, 1920
20. Hern, W. N.: Correlation of fetal age and measurements between 10 and 26 weeks of gestation. Obstet Gynecol, **63:** 26, 1984
21. Mercer, B. M., Skalar, S., Shariatmadar, A. et al: Fetal foot length as a predictor of gestational age. Am J Obstet Gynecol, **156:** 350, 1987
22. Platt, L. D., Medearis, A. L., DeVore, G. R. et al: Fetal foot length: relationship to menstrual age and fetal measurements in the second trimester. Obstet Gynecol, **71:** 526, 1988
23. Keis, T. and Vale, R.: Guidebook to the Extracellular Matrix and Adhesion Protein. Oxford: University Press, pp. 40–44, 1993
24. Bastos, A. L., Costa, W. S., Cardoso, L. E. et al: Collagen and elastic fibers of the penis in human fetuses with 28 weeks postconception. Eur Urol, **34:** 158, 1999
25. Sampaio, F. J. B., Pinto, J. L. F., Costa, W. S. et al: Quantitative analysis of smooth muscle fibers in corpus cavernosum of human fetuses. J Urol, **159:** 2226, 1998
26. Hsu, S. M., Raine, L. and Fanger, H.: Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem, **29:** 577, 1981
27. Beasley, S. W. and Hutson, J. M.: The role of the gubernaculum in testicular descent. J Urol, **140:** 1191, 1988
28. Wensing, C. J. G.: The embryology of testicular descent. Horm Res, **30:** 144, 1988
29. Hrabovszky, Z. and Hutson, J. M.: Capsaicin restores gubernacular contractility in TS rats. J Pediatr Surg, **34:** 1769, 1999
30. Bartlett, J. E., Washburn, T., Eddy, E. M. et al: Early development of the gubernaculum and cremaster sac in estrogen receptor knockout mice. Urol Res, **29:** 163, 2001
31. Scaffidi, A. K., Moodley, Y. P., Weichselbaum, M. et al: Regulation of human lung fibroblast phenotype and function by vitronectin and vitronectin integrins. J Cell Sci, **114:** 3507, 2001
32. Fentener van Vlissingen, J. M., van Zoelen, E. J., Ursem, P. J. et al: In vitro model of the first phase of testicular descent: identification of a low molecular weight factor from fetal testis involved in proliferation of gubernaculum testis cells and distinct from specified polypeptide growth factors and fetal gonadal hormones. Endocrinology, **123:** 2868, 1988
33. Kubota, Y., Nef, S., Farmer, P. J. et al: Leydig insulin-like hormone, gubernacular development and testicular descent. J Urol, **165:** 1673, 2001