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IS THERE A RELATIONSHIP WITH ANOMALOUS INSERTIONS OF THE DISTAL GUBERNACULUM TESTIS AND TESTICULAR ECTOPIA? ANALYSIS IN HUMAN FETUSES AND PATIENTS WITH CRYPTORCHIDISM

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ABSTRACT

Purpose: One of the most accepted theories for explaining testicular ectopia is the existence of multiple distal insertions of the gubernaculum testis. We studied the distal insertions of the gubernaculum in human fetuses and patients with cryptorchidism.

Materials and Methods: We studied 326 gubernacula in 163 human fetuses at 10 to 35 weeks of gestation and 133 gubernacula in 101 patients with cryptorchidism (mean age 6.4 years). With the aid of a stereoscopic microscope (2.5× magnification), the existence of distal insertions of the gubernaculum was investigated. Fisher's exact test was used to compare the 2 study populations.

Results: Of 326 fetal testes 224 (68.7%) were abdominal, 45 (13.8%) inguinal and 55 (16.9%) scrotal. In 1 fetus (0.6%) at 23 weeks of gestation both testes and both gubernacula were absent. Among 133 cryptorchid testes 17 (12.8%) were abdominal, 92 (69.2%) inguinal, 24 (18%) high scrotal and 3 (2.3%) evanescent. Of 324 fetal gubernacula present we found only 2 anomalous distal insertions in the pubopenile region (0.6%). All other distal insertions (99.4%) were at the usual location. Of 133 cryptorchid testes the 3 evanescent testes did not present visible gubernacula. Among 130 gubernacula analyzed all were inserted in the scrotal region.

Conclusions: The existence of anomalous insertions at the distal portion of the gubernaculum is rare in human fetuses and patients with cryptorchidism.

KEY WORDS: testis, cryptorchidism, embryo and fetal development, scrotum

The testis has its origin in the abdomen during the fetal period, migrating through the inguinal canal toward the scrotum between 15 and 28 weeks of gestation. Several theories have been proposed to explain testicular migration. The most accepted are increase in abdominal pressure; epididymis, spermatic vessel, vas deferens and inguinal canal development; stimuli originating from the genitofemoral nerve; hormonal and bioactive peptide stimuli with systemic or paracrine effects; and development of the gubernaculum.[1,2]

The gubernaculum testis seems to be the most important structure involved in testicular migration. The gubernaculum is a mesenchymal structure attached superiorly to the inferior pole of the testis and the epididymal tail (proximal or testicular portion), and attached inferiorly in the inguinal canal or the scrotum (distal or scrotal portion). The gubernaculum is formed by an abundant extracellular matrix enriched in glycosaminoglycans and mesenchymal cells, as well as fibroblasts and muscle cells.[1–4] The role of the gubernaculum in testicular migration is especially due to its ability to dilate and shorten.[1–4] The gubernaculum increases its volume primarily during the second trimester of gestation, when the testes pass through the inguinal canal, probably due to an increase in glycosaminoglycans concentration.[4,5] This increase seems to favor the passage of the gubernaculum through the inguinal canal.[1,3]

An important aspect is the difference between proximal (testicular) and distal (scrotal) portions of the gubernaculum. In experimental studies sections were made of proximal and distal portions of the gubernaculum, and the influence of these segments was observed in testicular migration.[2,6] In the group of animals with the distal portion of the gubernaculum sectioned no testis migrated to the scrotum, while in the group with the proximal portion sectioned 41% of the testes migrated to the scrotum.[6] These findings suggest that the distal portion of the gubernaculum has an important role in testicular migration.

Insertion of the proximal portion of the gubernaculum is altered in epididymal anomalies, which are frequently associated with cryptorchidism.[7–9] In one study of fetuses without congenital malformations or epididymal alterations, such as tail dysjunction or elongated epididymis, the proximal portion of the gubernaculum was attached to the testis and epididymis in all cases.[9] On the other hand, in undescended testes there was an increased incidence of paratesticular structure malformations accompanied by gubernacular attachment anomalies (27.8%) compared with the testes in normal fetuses. Also, a low incidence (2.75%) of epididymal abnormalities has been found in normal human fetuses.[10]

The point of insertion of the distal portion of the gubernaculum is one of the factors involved in testicular ectopia.[1,7,11] Previous studies have demonstrated that the distal portion of the gubernaculum can present 6 extensions—abdominal wall (interstitial), pubopenile, femoral, perineal, scrotal and contralateral hemiscrotum.[1,5,11] Some think that these branches of the distal portion of the gubernaculum exist at the beginning of fetal development and disappear during the process of testicular migration.[1,5,11] If any protraction of the distal portion persists, the individual could present with testicular ectopia.[11]

The point of insertion of the distal portion of the gubernac-

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ulum has already been described in human fetuses.[5] However, the existence of several branches of distal insertion is a controversial issue.[1,5,11] The distal insertion of the gubernaculum has also been analyzed in patients with cryptorchidism.[12] However, no published study compares the point of insertion of the distal gubernaculum in human fetuses to that of patients with cryptorchidism. In addition, there are few reports in the literature with a significant number of human fetuses in which the distal portion of the testicular gubernaculum has been studied. We analyzed the site of fixation of the distal portion of the gubernaculum during the human fetal period and in patients with cryptorchidism, exploring the presence of multiple distal insertions of this structure.

MATERIALS AND METHODS

We studied 326 testes in 163 fresh human fetuses and 133 testes in 101 patients with cryptorchidism undergoing orchiopexy. The committee on human research at our institution approved the investigation. All fetuses were well preserved, with no detectable congenital malformations. The fetuses were gestational age 10 to 35 weeks (corresponding to 12 to 37 postmenstrual weeks), estimated by the greatest foot length method.[13–15] After fetal classification the abdomen, inguinal canal and scrotum were opened to identify testes, epididymis and gubernaculum. The testis was regarded as abdominal when it was proximal to the internal ring, canalicular or inguinal when it was found between the internal and external inguinal ring, and descended or scrotal when it was inferior to the external ring. The path of the gubernaculum was carefully dissected with the aid of a stereoscopic lens with 2.5× magnification.

A total of 101 patients with cryptorchidism were analyzed between December 1999 and August 2002. Patient age was 1 to 15 years (mean 6.4), and the anatomy of the distal portion of the gubernaculum was assessed during routine orchiopexy. Cryptorchid testes were classified as being abdominal, inguinal or high scrotal.[14] Patients underwent conventional or laparoscopic orchiopexy, during which the path of the gubernaculum, as well as its point of distal insertion, was dissected.

We found that the distal portion of the gubernaculum could present 6 extensions, according to previous studies—abdominal wall (interstitial), pubopenile, femoral, perineal, scrotal and contralateral hemiscrotum (fig. 1).[1,3,5,11] Fisher's exact test was used to compare the fetuses and patients with cryptorchidism.[16]

RESULTS

Of 326 fetal testes 224 (68.7%) were abdominal, 45 (13.8%) inguinal, 55 (16.9%) scrotal and 2 (0.6%) absent. The 2 absent testes belonged to the same fetus at 23 weeks of gestation. Location of the fetal testes is shown in table 1. There were 178 testes (89 fetuses) at 17 to 26 weeks of gestation, of which only 5 (2.8%) had distal insertion in the scrotum. All others inserted in the inguinal canal (fig. 2).

Of 133 cryptorchid testes 17 (12.8%) were abdominal, 92 (69.2%) inguinal and 24 (18%) high scrotal. There was no ectopic testis. Of the 17 abdominal testes 3 (17.6%) were atrophic and 2 evanescent (11.8%). Of the 92 inguinal testes 1 (1.1%) was evanescent. Location of the cryptorchid testes is outlined in table 2.

Of 326 fetal gubernacula 2 (0.6%) were absent, and in 2 cases (0.6%) we found anomalous distal pubopenile insertions (fig. 3). Both anomalous distal insertions were observed in fetuses with abdominal testes. All of the remaining 322 gubernacula had a single distal insertion in the inguinal canal (262 [81.4%]) or scrotum (60 [18.6%]).

Of 133 cryptorchid testes 3 (2.3%) were evanescent and the gubernaculum was not identified. In the remaining 130 testes the gubernaculum inserted in the scrotal regions (high 61 [46.9%], low 44 [33.8%] and lateral 25 [19.2%]).

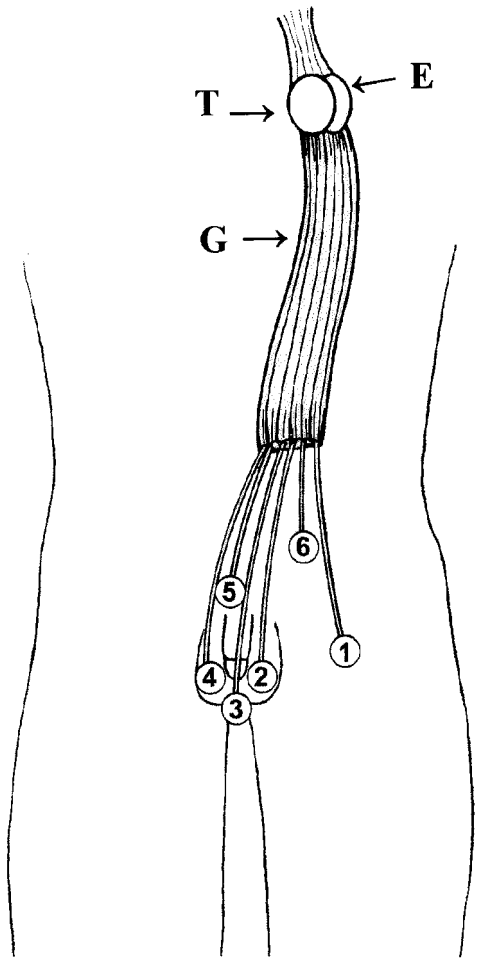

FIG. 1. Six extensions of distal portion of gubernaculum. *1*, Femoral. *2*, Scrotal. *3*, Perineal. *4*, Contralateral hemiscrotum. *5*, Pubopenile. *6*, Abdominal wall (interstitial). *T*, testis. *E*, epididymis. *G*, gubernaculum.

TABLE 1. *Position of fetal testes*

Region	No. Testes (%)
Abdominal	224 (68.7)
Inguinal canal	45 (13.8)
Scrotal	55 (16.9)
Congenitally absent	2 (0.6)
Total	326 (100)

DISCUSSION

The gubernaculum undergoes several structural modifications during the human fetal period and those modifications may be implicated in testicular migration.[1,4] 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 the gubernaculum becomes progressively more fibrous with low cellularity.[4] The remodeling of gubernacular connective tissue has been found to be rapid between 20 and 24 weeks of gestation, corresponding with previous observations that testicular passage through the inguinal canal at this time in gestation is likewise rapid.[9,17]

Recent studies of human fetuses have revealed histological differences between proximal and distal gubernacular regions.[4] Elastic fibers concentrate at the distal end of the

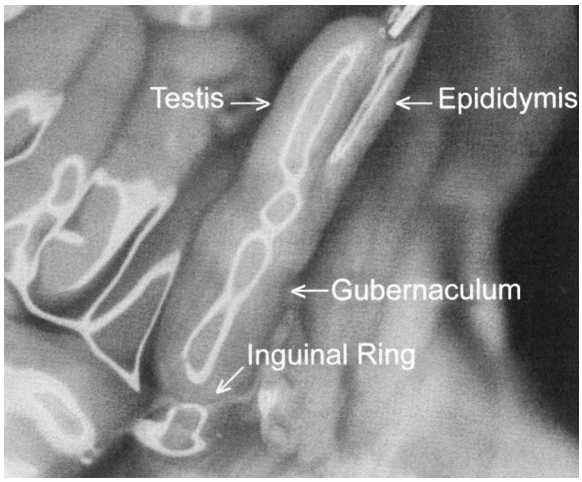

FIG. 2. Fetus at 20 weeks of gestation exhibits both testes in abdomen, with insertion of right gubernaculum at inguinal canal.

TABLE 2. *Location of cryptorchid testes*

Region	No. Testes (%)
Abdominal	17 (12.8)
Inguinal canal	92 (69.2)
High scrotal	24 (18)
Total	133 (100)

Of 17 abdominal testes 3 (17.6%) were atrophic and 2 (11.8%) evanescent. Of 92 inguinal testes 1 (1.1%) was evanescent.

FIG. 3. Fetus at 16 weeks of gestation with both testes (*T*) in abdomen, and 2 insertions of distal portion of right gubernaculum (*G*) in scrotum and in pubopenile region.

gubernaculum and appear only later in testicular descent.[4] After birth, and in individuals with normally descended testes, the gubernaculum differentiates into fasciae associated primarily with the testis and the epididymis. Also, in the early fetal period the presence of striated muscle cells has been demonstrated in the distal gubernaculum. The amount of muscle cell bundles decreases with fetal age, and during the entire gestational period they are located at the distal end of the gubernaculum.[4] In addition, during the entire fetal period when testicular descent occurs there is an overall small number of muscular cells in the gubernaculum.[4] It is speculated that these bundles of striated muscle might be the attachment fibers of the cremaster muscle.[1] Based on immunostaining of the gubernaculum with anti-smooth muscle α-actin antibody, it was verified that smooth muscle cells in the gubernaculum are restricted to the walls of blood vessels, and myofibroblasts should not be present in significant numbers.[4] Also, the striated muscle cells bundles at the gubernacular distal end have a low concentration and are mainly transversely oriented. These findings suggest that the human gubernaculum should not be capable of significant contraction, contrary to findings in rats.[18,19] Therefore, in humans if the gubernaculum pulls the testis down from the abdomen into the scrotum, it should be mainly a result of remodeling and consequent shortening of its connective tissue.[4]

The testis is considered ectopic when it lies outside its usual route of migration. Ectopia is rare, as it is estimated that approximately 5% of cryptorchid testes are ectopic.[7,11] Several theories attempt to explain testicular ectopia. The most accepted are failure in the mechanism of dilation of the inguinal canal by the gubernaculum that would free the testis during its migration, resulting in excessive mobilization to places outside its normal route; invasion of the gubernaculum by fasciae of the abdominal wall near the inguinal canal, which leads to an obstruction in the passage of the testis toward the scrotum, favoring its diversion to an ectopic place; and the existence of multiple distal insertions of the testicular gubernaculum, which would lead the testis to the primary sites of insertion, producing ectopia.[1,3,7,11]

The most common type of testicular ectopia is interstitial ectopia.[11] This condition is characterized by the testis being located on the surface of the external oblique muscle, in a superficial position (subcutaneous and easily palpable during physical examination). Some controversy exists regarding whether this testicular position should be considered ectopia.[7] In studies in which interstitial testes are considered ectopic a relatively high incidence of ectopia is reported.[12] On the other hand, in studies in which interstitial testes are not considered ectopic a low incidence of ectopia is found.[7] In this study we did not find interstitial testes in a high position. All of the interstitial testes were located close to the external inguinal ring (in a region known as the superficial inguinal pouch). We considered interstitial testes located close to the superficial inguinal ring as "high scrotal" (18% of our sample).

The most accepted theory to explain testicular ectopia is the existence of multiple distal insertions of the gubernaculum. According to this theory, proposed by Lockwood more than 100 years ago, the gubernaculum can present 6 points of distal insertion—scrotal, abdominal wall (interstitial), femoral, perineal, contralateral hemiscrotal and pubopenile, in descending order.[1,11] The multiple distal insertions of the gubernaculum are speculated to exist during the fetal period, and to vanish during the testicular migration process.[1,3,11] Pubopenile testicular ectopia is considered the rarest form.[11,20] However, in our sample the only 2 cases of anomalous insertion were located in the pubopenile region.

In previous studies of fetuses multiple distal insertions of the gubernaculum have not been observed.[3] In our study we did not find the 6 distal insertions simultaneously in any fetus. However, 2 gubernacula had anomalous insertions in the pubopenile region. This finding corresponds with the theory of the existence of multiple distal insertions in the end portion of the gubernaculum, at some point during fetal development, before its disappearance.[1,7]

The usual point of insertion of the gubernaculum during testicular migration is variable. Previous studies have shown that during the period before completion of testicular migration the distal portion of the gubernaculum is not firmly fixed in the scrotum. However, while the testis is in the abdomen the gubernaculum is firmly fixed to the inguinal canal.[3]

Heyns studied 306 fetal testes at gestational age 17 to 26 weeks, with the majority located in the abdomen, and observed that only 6 gubernacula (2%) had a distal fixation point in the scrotum.[3] In our sample during the same gestational period we found 178 testes, of which only 5 (2.8%) had distal insertion of the gubernaculum in the scrotum. This

finding corresponds to another study by Heyns et al, which revealed that the distal insertion of the gubernaculum when the testis was still situated in the abdomen was located at the inguinal canal in the majority of cases.[5]

The distal insertion of the gubernaculum is considered anomalous in more than 80% of cryptorchid cases.[12] These anomalous insertions are located in the region lateral to the scrotum or in the high scrotal portion. There are no reports of multiple distal insertions of the gubernaculum in patients with cryptorchidism. In our sample of 130 cryptorchid testes all gubernacula inserted in the scrotal region (46.9% high, 33.8% low and 19.2% lateral scrotum), and we did not find multiple distal insertions in any case.

CONCLUSIONS

The results of this study indicate that the majority of fetal testes located in the abdomen present distal insertion of the gubernaculum in the region of the inguinal canal. In addition, distal protractions of the gubernaculum were not observed in any patient with cryptorchidism, and were seen in only 0.6% of the fetal testes. Also, only 33.8% of the patients with cryptorchidism had the distal portion of the gubernaculum fixed in the lower scrotal region. Finally, the presence of protractions of the distal portion of the gubernaculum is rare after the first trimester of gestation.

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