

## PROXIMAL INSERTION OF GUBERNACULUM TESTIS IN NORMAL HUMAN FETUSES AND IN BOYS WITH CRYPTORCHIDISM

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### ABSTRACT

**Purpose:** We determine how the proximal gubernaculum testis is attached to the testis and epididymis in human fetuses, and compare these data with findings in boys who had undergone surgery for cryptorchidism.

**Materials and Methods:** We analyzed 280 testes and epididymides with the gubernacula of 140 well preserved, fresh human fetuses ranging from 10 to 35 weeks after conception with no detectable congenital malformations and 36 undescended testes of 28 boys 2 to 15 years old (mean age 6.8) who had undergone surgery for cryptorchidism. In both groups the different conformations of the relationship among the proximal gubernaculum, testis and epididymis were classified according to a system used for patients with cryptorchidism. In group A the gubernaculum is attached to the testis and epididymis, in group B the gubernaculum is attached only to the testis with a tail disjunction epididymal anomaly, in group C the gubernaculum is attached only to the testis with total disjunction of the epididymis, in group D the gubernaculum is attached only to the epididymal tail and in group E there are no attachments among gubernaculum, testis and epididymis.

**Results:** Of the 280 fetal testes studied 194 (69.2%) were in the abdomen, 38 (13.57%) in the inguinal canal and 48 (17.14%) in the scrotum. There were 277 cases (98.9%) in group A and 3 (1.1%) in group B. Of the 36 undescended testes analyzed 2 (5.6%) were abdominal and 34 (94.4%) were inguinal. There were 26 cases (72.2%) in group A, 8 (22.2%) in group B and 2 in group D.

**Conclusions:** In fetuses without congenital malformations or epididymal alterations, such as tail disjunction or elongated epididymis, the proximal portion of the gubernaculum was attached to the testis and epididymis in all cases. In undescended testes there was an increased incidence of paratesticular structure malformations accompanied by gubernacular attachment anomalies compared to the testes in normal fetuses.

KEY WORDS: testis, fetus, epididymis, cryptorchidism

The testis originates embryologically in the abdomen, and during the human fetal period it migrates to the scrotum through the abdominal wall. Many factors, either isolated or in association, have been proposed to be the causative agent of this descent, and the most commonly accepted are an increase in intra-abdominal pressure, hormonal stimuli from the placenta and testis, the development of testicular gubernaculum, vas deferens, epididymis and testicular vessels, and neural stimuli from the genitofemoral nerve.<sup>1–7</sup>

The role of the gubernaculum in testicular migration is thought to be of utmost importance. This elongated and cylindrical structure is composed of large amounts of extracellular matrix, which is particularly enriched in glycosaminoglycans and proteoglycans, and of mesenchymal cells, such as fibroblasts and muscle cells.<sup>8</sup> Some speculate that a shortening of the gubernaculum, possibly with the participation of the muscle cells, pulls the testis down into the scrotum.<sup>5,8,9</sup> Moreover, in the early phases of testicular migration, dilation of the gubernaculum occurs due to an increase in glycosaminoglycan synthesis, which has been demonstrated in pigs.<sup>10</sup> This dilation in turn expands the inguinal canal, thereby facilitating passage of the testis through this region.<sup>5,8,9</sup>

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The proximal portion of the gubernaculum is attached to the inferior pole of the testis and to the epididymal tail. Experimental studies on animals have indicated that when the gubernaculum is sectioned proximally, the testis does not migrate completely in the majority of the cases.<sup>11</sup> Also, the proximal gubernaculum is modified in many epididymal anomalies, such as elongated epididymis and epididymal disjunction, all commonly associated with cryptorchidism.<sup>12–16</sup>

The relationship among the gubernaculum, testis and epididymis has been analyzed in patients with cryptorchidism,<sup>12–15,17</sup> and the anatomy of the proximal gubernaculum has been investigated in human fetuses.<sup>8</sup> Nevertheless, to our knowledge no study has been performed on the occurrence of malformed epididymis and its relationship to the proximal gubernacular attachments in normal human fetuses. We determine how the gubernaculum is attached to the testis and epididymis in human fetuses, and compare these data with the findings in boys who underwent surgery for cryptorchidism.

### MATERIALS AND METHODS

We studied normal human fetuses and boys who had undergone surgery for undescended testes. The fetal sample consisted of 140 fresh male human fetuses 10 to 35 weeks after conception with 280 testes, epididymides and gubernacula. All fetuses were macroscopically well preserved and showed no signs of congenital malformations. Fetal age was calculated using the greatest foot length method.<sup>18–21</sup> The

abdominal and pelvic cavities were opened, and the position of the testes was determined. The testis was regarded as abdominal when it was proximal to the internal inguinal ring, canalicular or inguinal when it was between the internal and external inguinal ring, and descended or scrotal when it was inferior to the external ring.<sup>21</sup>

In both groups the different conformations of the relationship among the proximal gubernaculum, testis and epididymis were classified according to a system<sup>17</sup> used for patients with cryptorchidism. In group A the gubernaculum is attached to the testis and epididymis, in group B the gubernaculum is attached only to the testis with a tail disjunction epididymal anomaly, in group C the gubernaculum is attached only to the testis with total disjunction of the epididymis, in group D the gubernaculum is attached only to the epididymal tail and in group E there are no attachments among gubernaculum, testis and epididymis (see figure).

The group of patients with cryptorchidism included 36 undescended testes of 28 boys 2 to 15 years old (mean age 6.8). The anatomical relationship of the proximal gubernaculum was analyzed during routine orchiopexy. The initial analysis was made from the outside of the tunica vaginalis or from the outside the processus vaginalis if it was patent, after dissection of the cremasteric muscle fibers. Afterward, the anterior leaf of the processus vaginalis was opened and the different conformations of the relationship among the proximal gubernaculum, testis and epididymis were recorded and classed into groups A to E (see figure). The relationship between stage of development (fetuses as opposed to boys with cryptorchidism) and the occurrence of specific gubernacular-epididymal anatomical conformations (groups A through E) were evaluated by an R x C test of independence (G-test).<sup>22</sup> This design also allowed inferences regarding the significance of the incidence of anomalies in fetuses and boys.

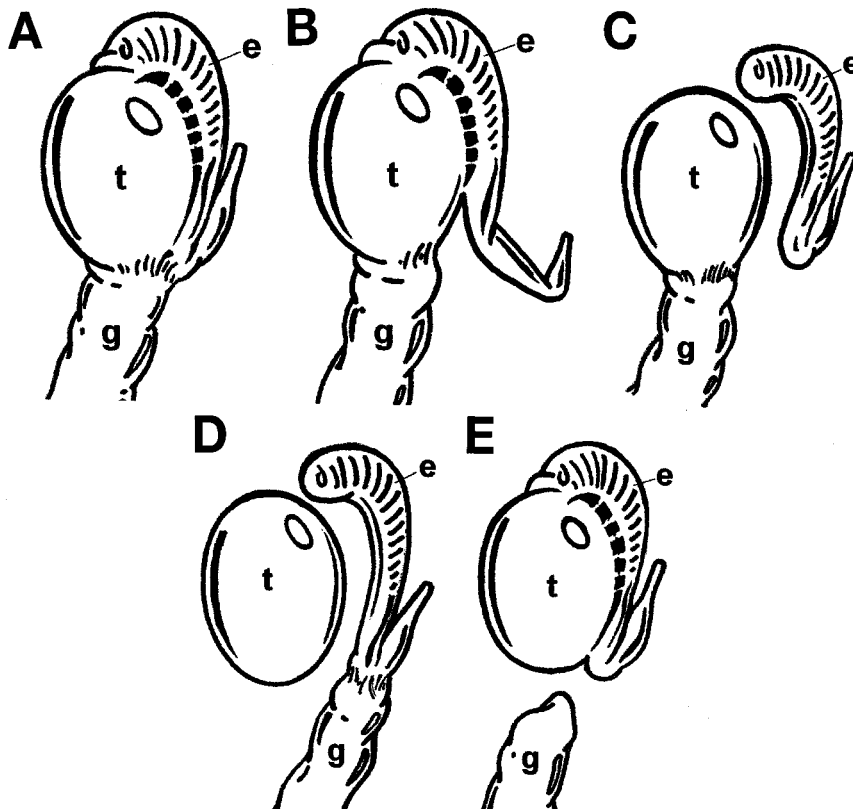
## RESULTS

Of the 280 fetal testes analyzed 194 (69.28%) were abdominal, 38 (13.57%) inguinal and 48 (17.14%) scrotal. The incidence of epididymal anomalies and the distribution of the gubernaculum-testis anatomical conformations in fetuses are shown in table 1. The majority (98.9%) were classified as group A (part A of figure) and 3 were in group B (part B of figure), and the anomalies were all unilateral. No groups C, D or E were found. In 1 of the 3 fetuses in group B the testes were in the abdomen and the gestational age was 24 weeks after conception. The other 2 fetuses were 29 and 35 weeks after conception and the testes were in the scrotum.

Of the 36 undescended testes analyzed 2 (5.6%) were abdominal and 34 (94.4%) were inguinal. The incidence of epididymal anomalies and the distribution of the gubernaculum-testis anatomical conformations in undescended testes are shown in table 2. Only 72.2% were classified as group A, 8 (22.2%) group B (unilateral in 6 and bilateral in 1) and 1 group D (bilateral, part D of figure). Using the test of independence the frequency of the different gubernacular-epididymal conformations was analyzed for fetal testes and testes of boys with cryptorchidism. The results indicated that the higher incidence of gubernacular-epididymal anomalies in the boys compared to the fetuses was significant ( $p < 0.001$ ).

## DISCUSSION

Structural epididymal anomalies are often associated with cryptorchidism with an incidence of 20% to 79%.<sup>12-16,23</sup> It has been reported that these abnormalities are especially associated with intra-abdominal testes.<sup>24</sup> A high incidence of epididymal abnormalities in association with a patent processus vaginalis, irrespective of testicular position, has also been reported.<sup>12,25</sup> In anomalies, such as epididymal disjunc-



Results of anatomical relationship among proximal gubernaculum (g), testis (t) epididymis (e). A, gubernaculum is attached to testis and epididymis (98.9% of normal fetuses, 72.2% of boys with cryptorchidism). B, gubernaculum is attached only to testis with tail disjunction epididymal anomaly (1.19% of normal fetuses, 22.2% of boys with cryptorchidism). C, gubernaculum is attached only to testis with total disjunction of epididymis (no cases). D, gubernaculum is attached only to epididymal tail (5.6% of boys with cryptorchidism). E, no attachments among gubernaculum, testis and epididymis (no cases).

TABLE 1. *Distribution of anatomical conformations in human fetuses*

|         | No. Fetuses (%) | No. Testes (%) |
|---------|-----------------|----------------|
| Group A | 137 (97.9)      | 277 (98.9)     |
| Group B | 3 (2.1)         | 3 (1.1)        |

TABLE 2. *Distribution of anatomical conformations in boys with cryptorchidism*

|         | No. Boys (%) | No. Testes (%) |
|---------|--------------|----------------|
| Group A | 20 (71.4%)   | 26 (72.2)      |
| Group B | 7 (25%)      | 8 (22.2)       |
| Group D | 1 (3.6%)     | 2 (5.6)        |

tion or elongated epididymis, the attachments among the proximal gubernaculum, testis and epididymis are altered. In most patients with cryptorchidism the proximal gubernaculum is attached only to the testis with no connections with the epididymis. However, in a few patients with cryptorchidism the gubernaculum is attached only to the epididymis or is not attached to the testis or epididymis.

The proximal portion of the gubernaculum is important for anchoring the testis to the region around the external ring, thus, guiding the testicular migration to the scrotum. Clarnette et al performed an experimental study in rats in which the proximal portion of the gubernaculum was sectioned.<sup>11</sup> After this procedure only 26 of 70 rats (37%) had normal testicular migration. In 19 rats the testes remained abdominal, while in 1 the testis was in the internal ring. In the remaining 24 rats testicular torsion occurred. This study indicates that the proximal gubernaculum is important not only for testicular migration, but also for limiting testicular mobility and, thus, preventing testicular torsion.

In a study of 44 patients with cryptorchidism an elongated epididymis was found in 42.5%.<sup>17</sup> Among the patients with an elongated epididymis attachment alterations in the proximal gubernaculum were found in 73.9%. In those remaining the attachments among the proximal gubernaculum, testis and epididymis were normal. In a previous study we analyzed 146 testes and epididymides of normal human fetuses between 10 and 36 weeks after conception and found a low incidence (2.75%) of epididymal abnormalities.<sup>26</sup> Similarly, a study of boys 1 month to 18 years old without cryptorchidism demonstrated a low incidence (3.5%) of epididymal abnormalities.<sup>16</sup>

It has been reported that epididymal abnormalities in cryptorchidism are more severe when associated with more cranially located testes.<sup>13,25</sup> Nevertheless, we found a low incidence of epididymal abnormalities (1.7%) associated with testes that were not in the scrotum during the fetal period in normal individuals. Of these testes 60.3% were in the abdomen and 21.2% were in the inguinal region.<sup>26</sup> Because the epididymal tail is normally attached to the gubernaculum, it has been speculated that epididymal abnormalities may interfere with normal testicular descent.<sup>12</sup>

In our current study we observed epididymal abnormalities in only 3 of 280 fetal testes. In the remaining 277 the proximal gubernaculum was attached to the inferior pole of the testis and to the epididymal tail. On the other hand, we confirmed that in undescended testis there is an elevated incidence of paratesticular structural malformations accompanied by gubernacular attachment anomalies compared to normal fetuses (27.8% and 1.1% of testes, respectively). These results indicate that, at least in part, such anomalies are related to impaired testicular migration. In conclusion, in fetuses without congenital malformations or epididymal alterations, such as tail disjunction or elongated epididymis,

the proximal portion of the gubernaculum was attached to the testis and epididymis in all cases.

Dr. Jayme Toledo provided some of the surgical data and Dr. Daniel Simplicio drew the anatomical diagrams.

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