Late ascended testes: is non-orthotopic gubernacular insertion a confirmation of an alternative embryological etiology?

B. Haid a,1, M.S. Silay b,1, A. Radford c,1, P. Rein a, B. Banuelos d, J. Oswald a, A.-F. Spinoit e,*,1

Summary
Introduction
Re-ascended testes account for a proportion of all undescended testes (UDTs); one main hypothesis relating to their etiology relates to a patent processus vaginalis peritonei. The aim was to investigate gubernacular insertion points in boys with late ascended testis as a possible guide to an alternative embryological etiology.

Patients and methods
Patients with proven ascended testes were recruited from four different pediatric urology centers between May 2016 and September 2017. All patients were evaluated regarding their gubernacular insertion during orchidopexy. The presence of accompanying patent processus vaginalis and the association between the epididymis and testis were also documented.

Results
Seventy-seven children (mean age 73.1 ± 41.2 months [range 18–176]) were enrolled into the study. A non-orthotopic gubernacular insertion was found in 96.1% (n = 74); 34.2% (n = 26) of these were located in the groin and 63.2% (n = 48), high within the scrotum. Figure A. An open processus vaginalis peritonei was found in 35.1%. Twelve patients (15.6%) had small, dysplastic appearing testis—epididymis dissociation. Boys with a higher insertion of the non-orthotopic gubernaculum (n = 48, groin) were operated earlier (mean age at surgery, 62.3 months) compared with those with a gubernacular insertion at a high scrotal site (mean age at surgery, 90.5 months; p = 0.004). Figure B.

Discussion
This study revealed that non-orthotopic gubernacular insertion is found in the vast majority of the ascending testis cases. Patent processus vaginalis was accompanying only 35.1% of all children and might be the cause of the ascending testis in this small subgroup of patients in line with the earlier reports [1]. In boys with ascending testes, in this population, the gubernaculum was very likely to insert non-orthotopically. In concordance with previous reports [2] and regarding the finding of an earlier age at surgery in boys with higher inserting gubernaculum, this could provide a logical explanation as to how these testes are initially palpable in the scrotum and then, during body growth are retracted to the groin.

Conclusion
In 96.1% of the patients, a non-orthotopic gubernacular insertion was found. This points to embryologic etiology, complying well with earlier reports and further underlining the critical importance of timely diagnosis and treatment for this group of patients.
References


Introduction

Undescended testis (UDT) is one of the most common conditions in pediatric urology and the single most common genital disorder diagnosed at birth. It is defined as a testis that has failed to descend to a scrotal position until birth or has re-asced from a scrotal position after birth; the latter is also referred to as acquired UDT [1].

The incidence of UDT for full-term boys accounts to around 2%, with wide variations dependent on birth weight and a far higher prevalence in preterm boys (up to 54%) [2]. Spontaneous descent occurs up until the 6th month of life in 35–43% of all affected children [2]. Given the orchidopexy rates of up to around 2–4% [3], the presumably important role of acquired UDT has been increasingly recognized recently [4,5]. Early studies dating from 1966 [4] describe the re-ascent rate as 69/4300 (1.6%) of observed boys, with further studies confirming these data [3]. Comparing the rates of non-descended testes by age 1 year (ca. 1%) with orchidopexy rates (2–3%), a similar incidence of testicular re-ascent is to be assumed [5].

Acquired UDT is reported to share histopathological features with the congenital UDT [6], putting the affected children presumably at the same risk for subfertility and testicular cancer compared with those with congenital UDT [6]. Concerning the risk of malignancy, however, the scrotal position in the early life might lead to a reduced frequency of later germ cell cancer in this particular group of patients—possibly even approaching the level of the general population [7]. Acquired UDT should be looked for actively and treated at the earliest possible after diagnosis; this, however, might be deferred owing to a lack of attention and less access to a regular physical exam in older boys as opposed to infants.

While there are theories as to the cause of the re-ascent involving the closure of a patent processus vaginalis (PPV) [8,9], an alternative explanation involves a dystopic gubernacular insertion being embryologically involved in testicular descent [10]. While a PPV is a very common finding in boys with and without ascended testes, it has been hypothesized before that gubernacular malinsertion could be a more consistent finding in boys with an ascending testis [11].

The developmental mechanisms driving testicular descent place the gubernaculum in a central position [10]. Possibly these mechanisms determine not only the path of testicular descent but also the final location of the testis. Gubernacular malinsertion, not ectopy, in a position too proximal could predispose to the postnatal finding of an UDT on the one hand. Additionally, it could be an etiological factor in acquired UTD with the testes being pulled up to the direction of groin during growth of the boy by the non-orthotopically inserted gubernaculum. Theoretically, the more proximal the non-orthotopic gubernacular insertion is situated, the earlier the testis concerned would be pulled up to the groin in course of the boy’s growth.

This study aimed to accurately evaluate the site of gubernacular insertion in boys with acquired UDT to validate the suspicion already formulated by Rabinowitz et al., in 1997 [11] that a non-orthotopic gubernacular insertion could be causative for a later testicular ascent during the growth of the boy.

Patients and methods

This study was organized by the Pediatric Urology Expert Group of the Young Academic Urologists of the European Association of Urology. Ethical approval was granted by the ethical committee of the Hospital of the sisters of Charity, Linz, Austria. Between May 2016 and September 2017, four different institutions registered for this prospective observational study (Linz, Ghent, Istanbul, and Feldkirch).

Only boys with a previously documented scrotal position of the concerned testis were included. Documentation of the previous scrotal position was mostly standard pediatric examinations or standardized reporting methods such as the ‘Mutter-Kind Pass’ (‘Mother-Child pass’) in Austria. Children who had previous inguinal surgery (hernia repair, hydrocele, and so on) which may cause iatrogenic ascending testis were excluded. In all included patients, testicular ascent was detected by a pediatrician (50.7%) or pediatric urologists (49.3%). All the indications for surgery were validated by experienced pediatric urologists (all certified Fellows of the European Board of Pediatric Urology/FEAPU).

For all patients, a standard inguinal orchidopexy was performed under general anesthesia. All patients were evaluated intraoperatively regarding the exact position of gubernacular insertion by putting the testis under gentle traction before dissection of the gubernaculum. Furthermore, the position of the testis is as follows: distal groin = at or distal to the outer inguinal ring; proximal groin = inside the inguinal channel; ectopic = outside the expected trajectory of testicular descent, either below the inguinal ligament or prepubic; and non-palpable = inside the inner inguinal ring. Possible pathologic gubernacular positions were predefined as Fig. 1. After skin incision, the outer inguinal ring and the aponeurosis of the external oblique muscle were prepared by dissection of subcutaneous tissues. The testis then was delivered and mobilized by careful dissection of the attached tissues, isolating the seminal strand cranially and gubernacular tissues caudally. By gentle pulling, the site of the main attachment of gubernacular tissues to the skin (‘insertion’) was identified and recorded. Fig. 2. The presence of accompanying patent processus vaginalis and the association between the epididymis and testis were also documented.

Data were stored on a Microsoft Excel® sheet after having been gathered by an anonymized, SSL encrypted online form. Data analysis was performed using GraphPad Prism software, to compare subgroups for categorical variables; Fisher’s exact test was applied.
Results

A total of 77 patients from four centers (58 Linz, 13 Gent, 5 Istanbul, and 1 Feldkirch) were included to this prospective study. All included boys had unilateral acquired UDT. Mean age at operation was 73.2 ± 41.2 months (range 18–176) months (mean age at surgery 90.5 months, \( p = 0.004 \)). Fig. 2.

During surgery, 81.9% (\( n = 63 \)) testes were found to be in the distal groin, 7.7% (\( n = 10 \)) in the high groin; 2.6% (\( n = 2 \)) were ectopic and 1.2% (\( n = 1 \)) were impalpable. One testis (1.2%) was found in the scrotum during surgery.

Gubernacular insertion was orthotopic in 3.9% (\( n = 3 \)) and non-orthotopic in 96.1% (\( n = 74 \)); 34.2% (\( n = 26 \)) insertion in the groin and 63.2% (\( n = 48 \)) insertion in the high scrotal.

The mean interval from the last documented scrotal position to surgery accounted to 26.6 months (range 1–164).

Discussion

This study revealed that non-orthotopic gubernacular insertion is found in the vast majority of the ascending testis cases. Patent processus vaginalis was accompanying only 35.1% of all children possibly being a cofactor of the ascending testis in this subgroup of patients.

This is in line with earlier publications suggesting at a causative role of a persistent processus vaginalis in testicular re-ascent [8,9,12]. Very recently, Wang et al. reported a higher rate of ascending testes after laparoscopic hernia repair, where the inguinal parts of the hernia sac remain intact compared the open procedure, where the sac is fully dissected. They assumed that open repair of an inguinal hernia could be a protective factor regarding secondary testicular re-ascent by a mechanism involving the hernia sac (i.e. PPV)—however, they concluded that the exact mechanism, even after previous inguinal surgery, remains unclear and should be further studied [13].

Alternatively, it has been proposed that also rigid residues of an already closed processus vaginalis (also termed Cloquet’s ligament) could be causal to a testicular re-ascent [9]. In this series, in each of 25 boys with secondarily ascended testes (\( n = 33 \)), a partially obliterated processus vaginalis was found. The conclusion is based on the surgeon’s impression that this structure was responsible for traction on and consequently re-ascension of the testis. All these boys, however, were operated by a scrotal approach, possible precluding a thorough evaluation of a possible patent processus at the level of the inner inguinal ring.

Whereas the impact of a persistent or fibrous processus vaginalis cannot be excluded based on the results, in the majority of the patients, a PPV was not present. Furthermore, the results comply very well with literature, where the incidence of a persistent processus vaginalis is described with an incidence of 17–25% in healthy children.
There is even evidence showing an open processus in 29% of completely unimpaired adults in course of postmortem examinations [16]. Another compelling theory on testicular re-ascent was formulated based on observations in patients with cerebral palsy: the authors (based on a sample of 25 patients with cerebral palsy who were compared with a group of matched controls) showed that while the testicular position changed in the control group (from mid to deep scrotal) with growth, it did not in the cerebral palsy group. In accordance with reports about high rates of acquired UDT in patients with cerebral palsy, the authors hypothesize about the role of a spasticity of the cremaster muscle as a possible cause for acquired UDT [17].

As to the role of the gubernaculum in acquired UDT, there is already literature available: Already in 1997, Rubinowitz et al. presented data of 21 boys with acquired UDT, theorizing that in the course of growth a 'nearly' normally inserting gubernaculum could ‘pull’ the testis from a previous scrotal position to the groin [11]. In this study, however, no standardized evaluation of gubernacular insertion was performed. In another series of 122 boys, however, the gubernaculum was described as normally inserting, albeit with no differentiated approach to the exact site of attachment [18].

Ascending testes might account for a relevant proportion of late orchidopexies of up to 45% [5]. While it is different from retractile testes, where the testis can easily be manipulated down to the scrotum, there is evidence for a history of retractile testes in up to 85% boys eventually concerned with acquired UDT according to literature [5].

In boys with ascending testes, in the population, the gubernaculum was very likely to insert non-orthotopically. This is concordant with previous reports [11] and could provide a logical explanation why these testes are initially palpable in the scrotum and then, during body growth are retracted to the groin. Subsequent to the most likely malinsertion of the gubernaculum in the distal groin or the high scrotal area, the most likely position of the testes in the suprainguinal pouch, as shown in the series and described in literature [18], seems logical.

Consequently, it was expected that those boys with a higher non-orthotopic gubernaculum in the groin should be prone to an earlier testicular ascent compared with those in whom the gubernaculum inserts more distally. The hypothesis was put to test by comparing the age at surgery of those with a gubernacular insertion in the groin to those with a gubernaculum insertion at a high scrotal site. The finding, that boys with a higher gubernacular insertion site are likely to be operated earlier (difference between means 28.21 months, \( \text{p} = 0.0043 \)), supports this hypothesis. The fact that a statistically significant difference can be found between those two groups of patients, although in many, the time between the last documented scrotal position of the testis and surgery was long (mean 26.6 months, range 1–164 months), further strengthens the value of these data.

Re-ascended testes have been shown to share the same histopathological features with primary UDT [6]. While this could be difficult to explain by an isolated mechanical factor as for instance cord structures fixed to a persistent processus vaginalis peritonei, a common, primary embryological etiology is a plausible explanation for shared effects on germ cell differentiation and eventually fertility.

As to possible etiological factors involved, the embryogenesis of the gubernaculum has to be reviewed. The first, transabdominal phase of descent is controlled by insulin-like hormone 3 and regression of the cranial suspensory ligament. The inguinoscrotal phase, between gestational week 25–35, is dependent on gubernacular extension involving enzymatic digestion of extracellular matrix [19]. The gubernaculum extends through the inguinal cannel and androgen, acting via the genitofemoral nerve, where calcitonin gene–related peptide is involved in the control of the direction of migration [20]. The gubernaculum, which is attached to different points during fetal development [21,22], contains contractile cells during the later embryonic stages and is remodeled to a

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<td>Age (mean, median) at the last scrotal position</td>
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fibrotic band fixing the testis to the scrotal wall in the final developmental phase [10,23]. Whether the reason for gubernacular malinsertion in the patients might be connected to a lack of factors involved in the growth of the gubernaculum, namely testosterone and calcitonin gene-related peptide, remains purely speculative [24]. Furthermore the use of the term ‘insertion’ should not be misinterpreted as an embryological fact, meaning that there is a fixed distal point of gubernacular attachment. Actually, embryological studies showed that the gubernaculum is attached to multiple points during fetal development [22]. The gubernacular remnant we identify by mechanical traction might merely be the rest of those structures, leaving many questions as to their final significance open. In this study, and in the study by Rabinowitz et al. [11], the observation of a gubernacular malinsertion was made only in patients with acquired UDT. It cannot, however, be excluded that a non-orthotopic gubernacular insertion might play a causative role also in primary UDT.

It has been repeatedly shown that re-ascended testes comprise a major group—up to 45%—in those boys who undergo surgery relatively late [5]. Also, diagnosis might be often deferred by too little knowledge about the possibility of a testis to ascend. This highlights the importance of further studies concerning its etiology and pathophysiology.

While the series is small, its strength lies in the prospectively collected data, originating from multiple centers and the same conclusion drawn from multiple examiners. The methodology of surgical preparation and visualization of the gubernaculum has been standardized by a detailed protocol before inclusion of the first patient. There is, however, a possible risk of individually different approaches to groin access and preparation that might potentially influence the visibility of the anatomy.

Another possible limitation lies in the risk of bias in diagnosing a re-ascended testis and differentiating it from a primary UDT that is inherent to this group of patients. As the underlying information on the previous testicular position in this series was selected very judiciously and the examiners are highly qualified, it is confident that the patients included herein represent true ascended testes.

**Conclusion**

In 96.1% of the patients, representing a well-documented cohort of children with testicular ascension, a non-orthotopic gubernacular insertion was found, whereas concordant with literature, only 35.1% had an open processus vaginalis peritonei. The finding that a higher gubernacular insertion correlates with a younger age at surgery is supporting the theory that the testes might be pulled up by a non-orthotopically inserting gubernaculum during growth. This might be interpreted as a hint toward an embryologic etiology for acquired UDT in the sense of a gubernacular malinsertion.

**Author statements**

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**Ethical approval**

Ethical approval was granted by the ethical committee of the Hospital of the sisters of Charity, Linz, Austria.

**Competing interests**

None of the authors declare any conflict of interest concerning this article.

**Data availability**

Underlying data for this study are available on request to the authors (bernhard.haid@ordensklinikum.at) at any time.

**References**


