Treatment of Congenital and Acquired Undescended Testes

Why How When

Clinical studies in adult men

Jocelyn van Brakel

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Treatment of Congenital and Acquired Undescended Testes

Why How When
Clinical studies in adult men

Behandeling van aangeboren en verworven niet ingedaalde testes
Waarom Hoe Wanneer
Klinische studies bij volwassen mannen

Proefschrift

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Voor Die MaMa

Geen dag uit mijn gedachte

Chapter 1
General introduction

Having two testes in the scrotum has been a symbol of manhood since old times. In the Middle ages only men with two scrotal testes, were able to candidate for papacy. After a scandal when a female pope gave birth to a baby during a papal procession in Rome the porphyry chair was produced (Figure 1). This chair had a cut out seat such that the elected candidate could sit suitably robed, while a junior cardinal could reach from behind and palpate the scrotum. If he chanted "duo testes bene pendulum" (he has two testes and they hang well) masculinity was confirmed and the candidate was eligible for the papacy. This excludes many boys since having undescended testis (UDT) is one of the most prevalent birth anomaly in boys. Testicular descent is a complex process and can go wrong at many stages during fetal development due to many different factors.

Normal testicular descent

During fetal development testes normally descend in two phases; the trans abdominal phase and the inguinoscrotal phase [(1)]. During the transabdominal phase, the gubernaculum enlarges under influence of Insulin-like 3 peptide (INSL3), whereas at the same time the cranial suspensory ligament regresses under influence of testosterone (Figure 2). This causes the testis to remain close to the position of the future inguinal canal as the abdominal cavity enlarges. This phase is normally completed at the 15^{th} week of gestation. In the inguinoscrotal phase, the gubernaculum with the attached testis migrates across the inguinal region into the scrotum [(1)]. This phase is androgen dependent and is completed by the end of the 35^{th} week of gestation.

Normal testicular development in early childhood

The testis has two compartments. The interstitial compartment in which male sexual hormones are produced and the tubular compartment consisting of seminiferous tubules in which spermatogenesis takes place. The most important cells in the interstitial compartment are Leydig cells which produce testosterone and INSL3. The tubular compartment contains Sertoli cells which are located on the basal membrane of the seminiferous tubule and germ cells which are located within the seminiferous tubule at different positions dependent on their stage within the spermatogenic process. During the first months after birth every boy goes through a so-called 'mini puberty' (Figure 3). During this hormone surge, which peaks at the age of 3 months, the number of Leydig cells and Sertoli cells increases with the increase of testosterone and Inhibin B [(3)]. Also the total number of germ cells increases in these three months. During mini puberty, in the first six months of life, gonocytes migrate from the center of the tubule towards the basal membrane which triggers the transformation into adult dark spermatogonia [(3,4)]. At the age of 3 to 4 years adult dark spermatogonia migrate back into the center of the tubule to mature into primary spermatocytes [(3-5)].

Normal testicular function in adults

The testis has two main functions e.g. producing testosterone and spermatogenesis.
Leydig cells are stimulated by luteinizing hormone, produced by the pituitary to produce testosterone. Testosterone in males is responsible for normal levels libido and energy, stimulating Sertoli cells, and normal bone density.
Sertoli cells are stimulated by follicle stimulating hormone, also produced by the pituitary, to stimulate spermatogenesis. From puberty spermatozoa are produced in the testis and are gaining their motility in the epididymis.

Background of undescended testes

Congenital undescended testes

2-8% of newborns have an UDT [8]. Congenital UDT are testes that have never reached a stable scrotal position. In a vast majority of these cases the second phase (inguinoscrotal) went amiss [3]. Testes can be palpable or impalpable. Nonpalpable testes are situated in the inguinal canal, intra-abdominally or are vanished testes. About 5% of operated UDT are localized intra-abdominally [9,10]. Palpable testes, at surgery, are situated suprascrotal (e.g. at or beyond the external ring, or in the superficial inguinal pouch) or high scrotal.
The cause of congenital UDT is not yet clearly known. Congenital UDT are associated with being born prematurely, birth weight < 2.5 kg, being small for gestational age, and having other genital abnormalities [6].

Other factors that might influence impaired testicular descent are described extensively in reviews from Virtanen and Toppari, Virtanen et al., and Hutson et al. and are summarized in table 1 [6-8]. It is most likely a combination of endocrine dysfunction, mechanical anomalies, and genetic factors.
In the first year most undescended testes at birth descend spontaneously. At one year of age 1-2% of boys remain to have one or two undescended testes [8]. This spontaneous descent occurs mostly in the first three months of life due to the increase of testosterone. After 12 months of age spontaneous descent is rare.

Acquired undescended testes

Nowadays, acquired UDT is a well-accepted distinct form of UDT [11-13]. The definition of an acquired UDT is a palpable testis that used to be in a normal scrotal position – as diagnosed and documented by a physician - and later has become entrapped outside of the scrotum. 1-3% of boys develop an acquired UDT during childhood [14,15]. The etiology and natural course for acquired UDT are still debated. Table 2 shows different hypotheses of the pathogenesis of

acquired UDT [16-19]. Figure 4 shows two of these possible hypotheses [16,17]. Undescended testes diagnosed after the age of 5 years are likely to be acquired UDT, supposing that these boys had a well set up routine medical programme during their first years of life [14]. The prevalence of acquired UDT is 1.2 to 2.2% between 6-year, 9-year and 13-year olds [14]. In contrast to congenital UDT, which are not expected to descend spontaneously after the age of 12 months, acquired UDT have a 57-76% change of descending spontaneously before or during puberty [20,21].

Retractile testes

A retractile testis is a testis which can alternately be found in the scrotum or groin region. It can be brought into a stable scrotal position but as a result of an active cremaster reflex moves up into the groin region [22]. The prevalence of retractile testis varies between 0.2 and 41% [23]. Retractile testes are usually considered a variant of normally descended testes but can become secondarily ascended over time [22].

Consequences of undescended testes

Fertility

Undescended testis is thought to be a developmental disorder with disruption of embryonal programming and gonadal development during fetal life [24]. The fact that the contralateral normally descended testis also shows histological deterioration strengthens the theory of a developmental disorder [5]. In addition to already abnormal testes at birth, in boys with undescended testis the number of germ cells decreases dramatically within the first two years of life especially after 6 months of age. Also the maturation of germ cells or gonocytes at 2-3 months of age into adult dark spermatogonia is delayed or defective [3,5]. Furthermore the transformation into primary spermatocytes is compromised, which occurs between 3 and 4 years of age [5]. In adult men with a history of treatment for bilateral undescended testis a paternity rate of 65% is found [25]. Paternity rate is 89.7% after treatment for unilateral UDT which is comparable with 93.2% found in the general population [25]. A group of experts estimated the chance of paternity in untreated bilateral UDT around 5% [26]. Both Sertoli cell function as well as Leydig cell function is found to be hampered in undescended testis [8].
For acquired UDT it is unknown what the consequences of being in a non-scrotal position are for fertility potential. It is suggested that these alterations are less severe since the crucial transformation of adult dark spermatogonia already occurred before the ascent [27].

Malignancy

Men with a history of undescended testis have a 4-5 fold increased risk of testicular cancer [28]. The pathogenesis for testicular malignancies in previous UDT lies within the impaired

transformation of gonocytes into adult dark spermatogonia. In UDT this transformation is interrupted or delayed followed by gonocytes undergoing apoptosis. It is hypothesized that the high temperature in undescended testis causes impaired transformation as well as prevention of apoptosis [4]. The remaining gonocytes could be the source for carcinoma in situ cells and therefore later testicular malignancies [29]. This is the reason why it is assumed that acquired UDT are not thought to have an increased risk for testicular malignancy. The transformation into adult dark spermatogonia already occurred and therefore no residual gonocytes that later form carcinoma in situ cell are thought to be present in acquired UDT later in childhood [2]. However this remains to be proven.
The problem with the current literature is that mostly no distinction is made between congenital and acquired UDT. Therefore the possible different consequences for outcome measures such as fertility and malignancy between congenital and acquired UDT are difficult to assess.

Treatment of undescended testes

Congenital UDT

Orchiopexy is the treatment of choice for congenital UDT. Over the years the recommended age at orchiopexy shifted from before puberty to 6 months of age aiming to minimize fertility problems at adult age [30]. This is based on several histological reports from biopsies taken at orchiopexy at different ages [31], and few studies which compared age of orchiopexy with rates of normal sperm count [8]. The idea is that ones the testis is in the scrotum postnatal germ cell development can proceed as normal [2]. However the literature remains inconsistent whether this has an effect on fertility. The Nordic consensus advises surgical intervention before the age of 12 months but after 6 months since spontaneous descent still occurs until 6 months of age [30]. A review showed that patients undergoing orchiopexy before puberty have a decreased risk for developing testicular malignancies [32]. Nowadays there is no place for hormonal therapy to induce testicular descent because of low success rates and high incidence of re-ascent of the testis [30]. The Dutch guidelines also advise surgery between 6 and 12 months of age for unilateral and bilateral congenital UDT [33].

Acquired UDT

The treatment for acquired UDT is still debated. In many countries it is unknown at diagnosis whether the UDT is a true acquired UDT or a missed congenital UDT. Therefore an orchiopexy mostly takes place at diagnosis. Two studies on the proportion of (pre) pubertal spontaneous descent in boys with acquired UDT found a prevalence of 57% and 71.4% for spontaneous descended testes [20,21]. For unilateral as well as bilateral acquired UDT, the Dutch guidelines advice to discuss the choice between immediate surgery and awaiting spontaneous descent

with parents and patient. In case of non-descent surgery should take place preferably before the age of 13 years [33]. Especially for bilateral acquired UDT the optimal timing of surgery is determined by weighing the cosmetic aspect against the complications of anesthesia and surgery as well as the spontaneous decent rate. In a decision analysis they could not find evidence that the earlier timing of surgery adds to improved fertility [26].

Retractile testes

Since retractile testis can ascend over time, surveillance is advised until after puberty [14]. The testis can spontaneously obtain a permanent scrotal position before or during puberty. Or the testis becomes an acquired UDT, which needs treatment accordingly. The last option is that the testis remains retractile in adulthood, which does not require treatment unless the testes cannot be brought down into the scrotum [22]. Retractile testes fall out of the scope of this thesis and will not be further discussed.

This thesis

Objective of this thesis

This thesis aims to elucidate differences between congenital and acquired UDT regarding natural course, fertility, endocrinology, and ultrasound anomalies. Literature available regarding acquired UDT and the differences between congenital and acquired UDT is rare. Most articles make no distinction between congenital and acquired UDT. In this thesis maximum effort was taken to distinguish between congenital and acquired UDT.

Outline of this thesis

In the first part of this thesis we describe surgical findings of acquired and congenital UDT. In **Chapter 2** we try to find an explanation for the phenomenon that some acquired UDT descend spontaneously while others need orchiopexy. **Chapter 3** reports on per operative clinical and surgical differences between congenital and acquired UDT.

The second part of the thesis attempts to answer questions regarding the influence of the timing of surgery and/or 'wait-and-see'- policy. We analyzed traditional fertility parameters (testicular volume, endocrinological values, and semen analyses) in adults with a history of congenital or acquired UDT in comparison with a healthy control group. **Chapter 4** tries to answer the following questions regarding congenital UDT; (1) What is the extent of fertility damage in men with a history of UDT in comparison with a healthy control group?; (2) What is the influence of age at orchiopexy on the fertility parameters in adult life?; and (3) Are there any differences in fertility parameters between men with unilateral and bilateral UDT?. Fertility parameters for acquired UDT are described in **chapter 5** and **6**. **Chapter 5** focuses on the possible impact of acquired UDT on fertility parameters in comparison with controls and men

with previously congenital UDT. Furthermore, it evaluates testicular function in men with acquired UDT after spontaneous descent or orchiopexy. In **Chapter 6** fertility parameters of men with acquired UDT and orchiopexy at diagnosis are compared with those of men who had a 'wait-and-see'-protocol for acquired UDT. Also the influence of age at orchiopexy, if operated upon, on fertility parameters in adult life was analyzed.

The third part of this thesis describes less traditional methods to evaluate endocrinological function and semen quality. **Chapter 7** examines differences in Leydig and Sertoli cell function between congenital and acquired UDT using Insulin-like peptide 3 (INSL3) and Anti -Müllerian hormone (AMH) serum levels. In addition, the influence of age at surgery in men with congenital UDT on these parameters as well as their association with spontaneous descent or orchiopexy in acquired UDT was examined. Also correlations of INSL3 and AMH with levels of other hormones of the pituitary-testis axis, semen parameters and testicular volume were investigated. **Chapter 8** analyzes the differences in sperm DNA damage between congenital and acquired UDT using Sperm Chromatin Structure Assay (SCSA). In addition, the influence of early or late orchiopexy in men with congenital UDT on sperm DNA damage levels, as well as the association with spontaneous descent or orchiopexy in acquired UDT was analyzed. Finally, the sperm DNA levels in men with a history of UDT attempting fatherhood were evaluated. **Chapter 9**, the fourth part of this thesis, reports extensively on different anomalies found by physical examination and scrotal ultrasound. We describe the anomalies of the undescended testis but also of their normal contralateral testis for congenital and acquired UDT in comparison with each other and a control group.
In the concluding fifth part of this thesis, **Chapter 10**, the main findings from this thesis will be reviewed and discussed in the general discussion. Recommendations for future research and implications for clinical practice are given. **Chapter 11** provides a summary of the contents of this thesis.

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Part I
Surgical findings in acquired and congenital undescended testes

Chapter 2

Surgical Findings in Acquired Undescended Testis: an Explanation for Pubertal Descent or Non-descent?

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Abstract

Aim:

Surgical findings were studied to find an explanation for the phenomenon that some acquired undescended testes (UDT) descend spontaneously whereas others need orchiopexy.

Methods:

In patients with acquired UDT spontaneous descent was awaited until at least Tanner stage P2G2. Orchiopexy was performed when a stable scrotal position had not been achieved by the end of follow-up.

Results:

Orchiopexy was needed in 57 of 132 cases (43%). In cases requiring orchiopexy, the difference in testis volume compared to the contralateral healthy testis was significantly larger than for spontaneously descended testes. 41 (72%) undescended testes were found in the superficial inguinal pouch; 16 (28%) at the external annulus. 26 of the 41 testes in the superficial inguinal pouch position (63%) could be manipulated preoperatively into a non-stable scrotal position; 15 could only reach the scrotal entrance prior to surgery. None of the 16 testes located at the external annulus could reach a scrotal position. Inguinal exploration in most cases revealed a fibrous string or a partially open processus vaginalis.

Conclusion:

The mobility of acquired UDT located within the external annulus is limited. It is mainly the fibrous string and the partially open processus vaginalis that prevent normal elongation of the spermatic cord with growth. These testes are unlikely to descend spontaneously. Acquired UDT lying in the superficial inguinal pouch can often be pushed down well below the scrotal entrance. We speculate that under normal hormonal stimulation at puberty, some of these growing testes may overcome the strength of the fibrous string in the spermatic cord and descend again spontaneously.

Introduction

It is generally accepted that congenital and acquired undescended testes (UDT) are 2 distinct entities although some etiological aspects are the same [1,2]. The aetiology, natural course, and treatment of acquired UDT are still subject of debate [1,3,4]. In a prospective study we followed boys with acquired UDT from referral until puberty; it appeared that 57% of testes descended spontaneously with a conservative wait-and-see approach [5]. The chance of spontaneous descent became higher with increasing age at referral, but other parameters evaluated were not associated with spontaneous descent or non-descent. Therefore, we focused on another important aspect, namely, the surgical findings collected during orchiopexy in those boys whose testes did not descend spontaneously. We aimed to answer the following question: can the surgical findings in acquired UDT elucidate why some of these testes descend spontaneously whereas others need orchiopexy?

Patients and methods

Patients and methods were described earlier [5]. In brief: from 1982 to 2004, boys with acquired UDT referred to our tertiary children's hospital were offered conservative treatment and were examined annually at least until puberty stage P2G2 according to Tanner [6]. Acquired UDT was defined as a palpable UDT that previously had a stable scrotal position, as documented at least once by the Regional Youth Health Care Institution, general practitioner or the referring hospital. Exclusion criteria were any ipsilateral or inguinal pathological clinical findings or previous ipsilateral inguinal surgery. The annual genital examination was performed by 2 senior authors (SdeMK-S and/or FH) with the patient in supine and in crossed-legged position. Since testis position may range, we registered its most caudal position after manipulation. Positions were categorized as inguinal region, at the scrotal entrance, unstable high scrotal or unstable low scrotal. Unstable was defined as moving to a non-scrotal position immediately after release. Retractile testes, i.e., testes that could be manipulated into the scrotum and maintained a stable scrotal position until induction of the cremaster reflex, were excluded.

Spontaneous descent was awaited until at least Tanner stage P2G2 (end of follow-up) [6]. Orchiopexy was performed when descent of the acquired UDT to a stable scrotal position had not occurred by the end of follow-up. Before operation, these patients were re-examined under general anaesthesia to exclude retractile testes and to assess most caudal testicular position.

In all cases, testicular volume was estimated by means of the Prader orchidometer [7] at the end of follow-up. In the cases with an unilateral acquired UDT and a normally descended

contralateral testis, difference in testicular volume at pubertal onset was calculated. Boys with bilateral acquired UDT and boys with unilateral acquired UDT with contralateral inguinal surgery were excluded from this analysis.

Mean testis volume differences in boys with spontaneously descended testes and boys who needed orchiopexy were compared using the t test. SPSS®, PASW Statistics 17.0 was used to analyse the results and a *p*-value ≤ 0.05 was considered to be statistically significant [5].

The most relevant anatomical findings of the boys who required surgery were registered as follows:

Intraoperative position

At the external annulus: lying in or just outside the external annulus underneath Scarpa's fascia and caudal to the still unopened inguinal canal.

In the superficial inguinal pouch: lying on the aponeurosis of the external abdominal muscle under Scarpa's fascia and cranio-lateral to the external annulus.

Processus vaginalis

Closed: processus vaginalis has been obliterated to a fibrous string.

Small open: small open communication between the peritoneal cavity and that part of the processus vaginalis immediately surrounding the testis that always remains open, the tunica vaginalis.

Wide open: the testis can move freely in the open processus vaginalis, ranging from the most caudal position to intraperitoneal position.

Epididymal anomalies

Normal: caput and tail are firmly attached to the testis, the epididymis body is either complete attached to the testis or separated from the testis.

Partial separation: the epididymis extends further than the length of the testis with only the head normally attached.

Complete separation: there is a macroscopic, total separation of epididymis and testis.

Long loop: the epididymis extends in a caudal direction, sometimes reaching the scrotum.

During surgery only orchiopexy was performed and no biopsy was taken in any of the patients.

Ethics

The study was reviewed and approved of by the Medical Ethical Review Committee of the Erasmus Medical Centre in Rotterdam. Informed consent was obtained from all patients who agreed to participate in the study.

Results

Follow-up period

Results up until the end of follow-up were published previously [5]. 109 boys with 135 cases of acquired UDT were followed up at least until puberty stage P2G2. Mean age at referral was 8.9 ± 2.9 years (range: 1-14.9 years). Mean follow-up was 4.5 years (range: 0.3-12.1 years). 2 boys were excluded from analysis including one with unilateral UDT who was lost to follow-up, while orchiopexy was requested by the parents of a boy with bilateral UDT. In the remaining 132 testes (82 unilateral and 50 bilateral), the previous scrotal position had been documented at least once for 53 testes, twice for 39 and 3 times or more for 40. Spontaneous descent to a stable scrotal position was noted for 75 testes (57%).

Thus, orchiopexy was performed for 57 acquired UDT (27 left and 30 right-sided) in 45 boys. Mean age at orchiopexy was 12.7 years (range: 5.2-15.8 years). In 47 testes this took place after Tanner stage P2G2; in 10 testes (8 unilateral and 1 bilateral) before puberty. The predominant reasons for earlier operation were inguinal complaints or a newly detected, clinically present, ipsilateral inguinal hernia [5]. The surgical findings of these 10 testes operated before puberty made a spontaneous descent very unlikely [5]. The total number of documented scrotal positions of the testes and the most caudal testis position at referral did not differ significantly between spontaneous descended testes and the testes that needed orchiopexy (Table1). Moreover, no association was found between the total number of documented scrotal testes position and most caudal testicular position at referral in either of the groups (p=0.361 and p=0.164 for the spontaneous descent group and the non-descent group, respectively).

Testicular volume differences were calculated for 63 of the 82 boys with unilateral acquired UDT. Excluded from this analysis were 19 boys (19 unilateral UDTs), including the above mentioned 8 who underwent orchiopexy before puberty and 11 with either previous contralateral orchiopexy for congenital UDT (n=9) or inguinal hernia (n=2). Of 17 testes that needed orchiopexy 12 (71%) had a volume more than 1 ml smaller than their healthy contralateral testis. This was noted in only 18 (39%) of 46 testes which descended spontaneously (chi-square: p=0.053) [5]. The difference in testicular volume of spontaneously

descended testes was significantly smaller than that of testes which needed orchiopexy (mean ± SEM: -1.5 ± 0.37 ml and -3.9 ± 0.97 ml, respectively t-test $p = 0.030$) [5].

For 3 testes the most caudal position preoperatively was in the inguinal region, for 28 at the scrotal entrance, and 26 could be manipulated into an unstable scrotal position.

Intraoperative findings

The intraoperative testicular position of the 57 testes that needed orchiopexy was the superficial inguinal pouch for 41 testes (72%), while the other 16 (28%) were located at the external annulus.

We created cross-sectional tables to identify any relationships between the intraoperative testicular position and other differing surgical findings. Cross-referencing the testis position at surgery using the most caudal testicular position preoperatively provided information about the mobility of the acquired UDT (Table 2). 26 (63%) of the 41 testes lying in the superficial inguinal pouch could be manipulated into an unstable scrotal position, whereas the other 15 could only reach the scrotal entrance. On the other hand, none of the testes located at the external annulus could reach a scrotal position: 13 (81%) of the 16 testes were moveable to the scrotal entrance; the other 3 were only palpable in the inguinal region.

Table 3 gives the intraoperative testicular position in relation to the condition of the processus vaginalis. In 33 of the 57 cases (58%) – notably when the testis was located in the superficial inguinal pouch – the processus vaginalis was obliterated to a fibrous string. Inguinal exploration rarely revealed a wide open processus vaginalis.

With regard to epididymal anomalies in relation to intraoperative testicular position (Table 4), 4 of 16 testes situated at the annulus externus showed epididymal anomalies, vs. 3 of 41 testes in the superficial inguinal pouch.

Discussion

The natural course and treatment of acquired UDT is a source of dispute in the Netherlands [3]. It is the Youth Health Care Centers task to register each boy's testicular position from birth to puberty. The Youth Health Physicians have noted that previously scrotal testes that had ascended would often return into the scrotum again. In their opinion a 'wait-and-see' approach until puberty is preferable to referral for surgery at presentation. On the other hand, most (paediatric) surgeons and urologists assume that, despite the likelihood of spontaneous descent at puberty, immediate orchiopexy could possibly be advantageous in terms of fertility,

as is assumed in congenital UDT [8]. In the study presented here, more than half of acquired UDT indeed descended spontaneously before the onset of at least Tanner stage P2G2.

To our knowledge only one other long-term follow-up study of boys with acquired UDT has been published: it reported a 76% spontaneous descent rate vs. 57% in our study [9]. As the study designs of both studies are largely comparable, this discrepancy could be the result, in part, of small differences in methodology, such as the formulation of exclusion criteria.

The present study focused on the question whether surgical findings could indicate the likelihood of a spontaneous descent of acquired UDT. A valid answer to this question cannot be made without insight into the aetiology of acquired UDT.

Atwel has proposed that it is matter of partial absorption of the persistent open processus vaginalis into the partial peritoneum during growth of the boy [10]. In 7 cases in our study there was a wide open processus vaginalis. Tanyel hypothesized that congenital and acquired UDT share the same aetiology [2]. The process of obliteration of the processus vaginalis by apoptosis is hampered in congenital UDT; furthermore, if initial descent has occurred normally because the process of programmed cell death is more subtle but the process persists, the testis may ascend later in time. This hypothesis is largely in line with Clarnettte et al. who suggested that a persisting fibrous remnant of the processus vaginalis prevents natural elongation of the spermatic cord during growth [11]. The last 2 hypotheses seem more likely to explain the aetiology of acquired UDT since this was indeed the case in the large majority of testes in the present study. The 58% incidence rate of completely obliterated processus vaginalis is in accordance with the 57-70% rates found in other studies [12,13]. Although the above mechanisms are very likely to cause acquired UDT, they do not yet explain why some of these testes can descend spontaneously.

Epididymal anomalies occur in 36-79% of undescended testes [14]. However, most studies reporting these anomalies neither define normal epididymis nor are they focusing exclusively on acquired UDT. In the present study epididymal anomalies occurred in only 7 of the 57 cases (12%). There are 2 explanations for this low incidence. First, we classified anomalies as a separation of caput and/or cauda epididymis from the testis or a long looping epididymis. A widened mesentery between the body of the epididymis was considered normal in line with other studies [15,16]. Secondly, since acquired UDT by definition have descended normally at an earlier stage, it is plausible to expect fewer anomalies in comparison to congenital UDT. To our knowledge, only one study by Guven and Kogan reports on epididymal anomalies in acquired UDT in compared to congenital UDT [17]. In that study 28% of acquired UDT had epididymal anomalies, compared with 46% of patients with congenital UDT who were operated late due to parental delay or late referral.

With regard to mobility, 16 testes located at the external annulus at surgery could never be brought down further than the scrotal entrance. It is unlikely that these testes will descend spontaneously, seeing that the fibrous remnant or (partially) open processus vaginalis prevents normal elongation of the spermatic cord. On the other hand, 26 of the 41 testes located in the superficial inguinal pouch could be brought down to an unstable scrotal position before operation. The superficial inguinal pouch, first described by Sir Denis Browne in 1938 [18], is a space situated between the Scarpa's fascia and the external oblique muscle and runs parallel to the inguinal canal. Browne proposed that the so-called 'high retractile testis' remains habitually remains and moves freely in this pouch, and does therefore not descend during childhood. It can, however, be pushed down well below the external ring, over the pubic bone, and into a high scrotal position. Spontaneous descent to a stable scrotal position may occur when the testis reaches adult size and weight. Brown in fact defined what we now accept as an ascending testis.

We speculate that acquired UDT, with a range of movement from the superficial inguinal pouch to an unstable scrotal position, can descend at puberty if massive enough to overcome the strength of the fibrous remnant in the spermatic cord. This supposition is supported by the fact that in the case of spontaneously descended testes the difference in volume to the contralateral healthy testis is smaller than in the case of testes which remained undescended and required surgery.

There are several limitations in this study. First, for obvious reasons, we have no insight into the anatomy of spontaneously descended testes. Second, in 53 boys a previous stable scrotal position was documented only once. Therefore, it cannot be excluded that these boys had congenital undescended testes. If so, it seems reasonable to assume that these boys would be more frequently present in the operated group than in the spontaneous descent group since congenital UDT rarely descend spontaneously after the first year of life [19]. This, however, is not the case in our study as shown in Table 1. Moreover, the most caudal position of the testis at referral did not differ between the 2 groups and was at a relatively low position in both groups of acquired UDT (Table 1). Since Meij-de Vries et al. [12] showed that testis position in congenital UDT is different from testis position in acquired UDT and that congenital UDT are located in a higher position compared to acquired UDT it seems unlikely that boys included in our study had congenital UDT.

The key question remains if a 'wait-and-see approach' is a better treatment option than surgery of acquired UDT at presentation [1]. To answer this question long-term follow-up studies are necessary to determine hormonal functioning, fertility potential, and cancer risks in three different groups of boys with acquired UDT: spontaneous descent at puberty, surgery immediate after diagnosis, or surgery delayed until puberty.

Conclusions

Failure of the spermatic cord to elongate with growth of the boy is mainly caused by the presence of the fibrous string or incomplete open processus vaginalis. Acquired UDT located within the external annulus have limited mobility and are unlikely to descend spontaneously.

Acquired UDT lying in the superficial inguinal pouch position can often be pushed down well below the scrotal entrance. Therefore, we speculate that under normal hormonal stimulation at puberty some of these growing testes can overcome the strength of the fibrous string in the spermatic cord and descend again spontaneously. The smaller volume difference between the undescended testes and their healthy contralateral testes in the group with spontaneous descent supports this assumption. Unfortunately, it is impossible to confirm this hypothesis since the anatomy of testes which descend spontaneously remains unknown.

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Chapter 3

Different surgical findings in congenital and acquired undescended testes.

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Abstract

Objective

To compare surgical findings in congenital and acquired undescended testes (UDT).

Patients and methods

A review of 139 boys with a 158 congenital UDT and 69 boys with 84 acquired UDT was performed. The most caudal testicular position preoperatively, testis position at surgery, patency of the processus vaginalis, and epididymal anomalies were prospectively recorded.

Results

In the congenital group, orchiopexy had been performed at median age (range) 4.9 (1.5-14.6) years, while the median age (range) in the acquired group was 11.9 (3.8-23.3) years. Preoperatively, only congenital UDT were found not palpable or emergent inguinal, while only acquired UDT could be manipulated in an unstable scrotal position. In comparison with congenital UDT, acquired UDT were significantly more often located at the scrotal entrance, 27/158 vs. 32/84 respectively ($P < 0.001$). At surgery anorchia, vanished testis or testes lying intra-abdominally were only registered in the congenital UDT group. Also 37/158 congenital UDT were located in the superficial inguinal pouch vs. 52/84 of the acquired UDT ($P = 0.04$). In congenital UDT the processus vaginalis was wide open in 74/158, while in acquired UDT the processus vaginalis was closed in 46/84 ($P < 0.001$) and small open in 26/84 ($P = 0.04$). Epididymal anomalies were more often seen in the congenital UDT group (37%) than in the acquired group (11%).

Conclusion

Most caudal position of congenital UDT after manipulation before surgery was at the scrotal entrance. These testes were frequently associated with epididymal anomalies and wide open processus vaginalis. This was in contrast to acquired UDT, which can often be pushed down

well below the scrotal entrance and are more likely to be situated in the supraficial inguinal pouch, with a normal epididymis and closed processus vaginalis.

Introduction

Undescended testes (UDT) are one of the commonest genital abnormalities in boys. Two types are distinguished: congenital and acquired UDT [1,2]. 1% of all boys will have a testis that has not yet descended by 1 year of age [3]. Congenital UDT are testes that have never reached a stable scrotal position. Acquired UDT are testes that used to be in a scrotal position – as diagnosed by a physician – and later have become entrapped outside the scrotum. This is seen in 1-3% of boys during childhood [4]. Much is known of the aetiology, natural course and treatment of congenital UDT, but these matters are less clear for acquired UDT and still debated. Furthermore, even less is known about anatomical differences between the two types of UDT. Several studies have examined anatomical findings in patients with UDT [5-19]. However, most of these studies did not distinguish between congenital UDT and acquired UDT. The aim of the study reported here was to objectify possible clinical and surgical differences between the two types.

Patients and methods

Boys included in this study had at prepubertal age been referred for UDT to the Erasmus University MC-Sophia Children's Hospital, Rotterdam. The surgical findings were registered in a standardised form. Previous testicular positions were retrospectively collected from medical documentation of the Regional Youth Health Care Institution, the general practitioner or hospital file to diagnose either congenital or acquired UDT.

Congenital UDT group

These boys participated in a placebo-controlled study of luteinising-hormone-releasing-hormone (LHRH) nasal spray between 1982 and 1985 [20]. They had been operated on by the same paediatric surgeon (FH) if LHRH did not achieve a permanent stable scrotal position.

Acquired UDT group

Acquired UDT is defined as a palpable UDT that had previously descended into a stable scrotal position. At least one medical document had to confirm previous stable scrotal position. Patients referred for acquired UDT between 1982 and 2004 were treated conservatively and examined annually at least until puberty stage P2G2 according to Marshall and Tanner [21]. If spontaneous descent did not occur, operation followed.

Definition of terms

Preoperative location

The most caudal testicular position found after admission and confirmed following induction of anaesthesia was classified as (i) not palpable; (ii) emergent inguinal: testis intermittently palpable; (iii) inguinal region; (iv) scrotal entrance; (v) unstable scrotal – testis replaced in non-scrotal position immediately after release.

Boys with retractile testes, i.e. testes that could be manipulated into the scrotum and maintained a stable scrotal position until induction of the cremaster reflex, were excluded.

Intraoperative location

The testicular location at the time of surgery was classified as: (i) absent – either true anorchia or "vanished testis"; (ii) intra-abdominal; (iii) inguinal canal; (iv) external annulus; (v) Superficial inguinal pouch.

Processus vaginalis

The condition of the processus vaginalis was classified as: (i) closed – obliterated to a fibrous string; (ii) small open – small open communication between the peritoneal cavity and tunica vaginalis; (iii) wide open – testis can move freely within the processus vaginalis.

Epididymis

The condition of the epididymis was classified as: (i) absent; (ii) partially separated from testis; (iii) complete separated from testis; (iv) long loop epididymis and vas.

Statistical analysis

Data were analysed with SPSS, version 17.0. (SPSS Inc., Chicago, IL, USA). Variables were tested for power to distinguish between congenital or acquired UDT. For preoperative testis position an univariate linear regression analysis was done, and for the intraoperative findings (intraoperative testis position, condition of processus vaginalis and epididymal anomalies) a multivariate analysis. $P < 0.05$ were considered statistically significant.

Ethics

The study was reviewed and approved by the Medical Ethical Review Committee at Erasmus University Medical Centre in Rotterdam (MEC number 2004-206).

Results

We analysed findings in 139 boys with congenital UDT (19 bilateral and 120 unilateral) and 69 boys with acquired UDT (15 bilateral and 54 unilateral). In the congenital group, orchiopexy had been performed after diagnosis at median age (range) 4.9 (1.5-14.6) years. Median age (range) in the acquired group was 11.9 (range 3.8-23.3) years. In 74 cases orchiopexy was performed after Tanner stage P2G2; in 10 before puberty. Most important reasons for earlier operation were inguinal complaints and a clinically present ipsilateral inguinal hernia.

Table 1 shows the characteristics of the two groups. Relevant differences between congenital and acquired UDT can be summarized as follows.

In 27 boys with unilateral congenital UDT the testis was preoperatively not or intermittently palpable. At surgery of these boys 15 testes were absent, 4 were located intra-abdominally, and 8 were lying in the inguinal canal. Acquired UDT were always palpable preoperatively. The most caudal position of 66% of congenital UDT was in the inguinal region; most acquired UDT (54%) could be manipulated into an unstable scrotal position. Testes found at the scrotal entrance were significantly more often acquired UDT (odds ratio [OR] 0.06; 95% Confidence Interval (95% CI) 0.02 – 0.14, $P < 0.001$). The acquired group had more often testes situated in the superficial inguinal pouch (OR 0.48; 95% CI 0.24 – 0.96, P 0.04).

In the congenital group the patency of the processus vaginalis was more often small or wide open, i.e. in 78% vs. 45% in the acquired group. Acquired UDT had significantly more a closed or small open processus vaginalis (Table 1). Correlations between the patency of the processus vaginalis and the intraoperative positions are shown in Table 2.

Epididymal anomalies were more often seen in the congenital UDT group than in the acquired group, 37% vs. 11% respectively. Table 3 shows the condition of the epididymis in relation to intraoperative testis position. The higher the intraoperative testis position, the higher is the number of epididymal anomalies. In both groups epididymal anomalies were associated with a patent processus vaginalis, especially a wide open processus vaginalis (Table 4).

Discussion

This study aimed at objectifying anatomical differences between congenital and acquired UDT observed at surgery. First we need to address the nature of the two study groups. The congenital group received LHRH nasal spray prior to orchiopexy. It is very unlikely that this hormonal treatment influenced the surgical findings because we found no evidence of stimulation of the hypothalamo-pituitary-gonadal axis and our data did not support the presence of hormonal abnormalities in the cryptorchid boys [20]. Following international recommendations, boys with congenital UDT are operated on before 1 year of age [22].

In The Netherlands the natural course and treatment options in acquired UDT are still being debated [2,23]. Our acquired UDT study group consisted of boys in whom spontaneous descent failed to occur, mostly until the onset of puberty. Therefore the median age of this group is higher than that of the congenital group. However, the key question here is whether a 'wait and see approach' until puberty – with surgery if the testis does not spontaneously descend – is to be preferred over surgery immediately following diagnosis [24,25]. This question falls outside the scope of this paper, but nevertheless we would recommend long-term follow-up studies comparing fertility potential and hormonal functioning between the two approaches.

Apart from differences in preoperative positions, there were indeed differences in surgical findings, supporting the idea that these two types are distinct entities. In the congenital group the most caudal position of the testes before surgery was mainly outside the inguinal canal, but no further than the scrotal entrance. In the acquired group, however, most testes could be manipulated to the scrotal entrance or to an instable scrotal position. Unfortunately, a similar study by Meij-de Vries et al. [15] does not document preoperative testes positions. The distribution of intraoperative testis positions significantly differed between the two groups. In the congenital group significantly more testes were lying in the inguinal canal than was the case in the acquired group. This was also reported by Meij-de Vries et al. [15], whose study, however, distinguished slightly different intraoperative testis locations. Also in other publications related to acquired UDT, the position of ascending testes at surgery was more likely to be in the superficial inguinal pouch [10,16,18].

Earlier studies found a patent processus vaginalis in 44-93% of patients with UDT [5,8,11,14]. However, these studies did not distinguish between congenital and acquired UDT and commenting on these rates is of little use because processus vaginalis status of normal boys is unclear [8,14]. We found the processus vaginalis was more often patent in congenital UDT, notably wide open, than in acquired UDT. To our knowledge three studies clearly distinguishing between congenital and acquired UDT present data on the patency of the processus vaginalis [10,15,19]. One reports similar results but that does not distinguish between small open and wide open processus vaginalis [15]. The second found fewer cases of patent processus vaginalis in the congenital group than in the acquired group. They suggested that a

patent processus vaginalis could explain the late descend or that testes with a patent processus vaginalis might be more at risk to ascent, however, to our knowledge this is the only study with these findings and different opinion [10]. The third also specifies whether the processus vaginalis is small open or wide open and findings are largely comparable with ours [19]. Furthermore, Clarnette et al. [6] found a closed processus vaginalis in all acquired UDT, and two other studies – reporting only on acquired UDT – found an incidence of closed processus vaginalis of 45% and 48%, respectively [16,18].

The incidence of epididymal anomalies in boys with UDT was reported to be higher than in boys with a hydrocele/hernia without cryptorchidism [5,7,9,11]. In our study more epididymal anomalies were seen in congenital UDT than in acquired UDT, 31% vs. 11% respectively. In the literature, proportions of boys with UDT showing epididymal anomalies range widely [5,7,9-14,17]. For example, in an overview by Elder they vary from 36 to 79% in boys with UDT [7]. There are two explanations for this wide range. First, most authors did not define the normal epididymis. We classified separation of caput and/or cauda epididymis from the testis or a long looping epididymis as anomalies. A completely attached or a widened mesentery between the body of the epididymis was considered normal, in line with Turek et al. [26]. Second, most studies make no distinction between the two types of UDT. Since acquired UDT by definition have descended normally at an earlier stage, it is plausible to expect fewer anomalies than in congenital UDT, as in our study. Different patient selection could thereby cause this wide range. To our knowledge, only one other study also compared epididymal anomalies in acquired UDT and in congenital UDT [10]. In that study 28% of boys with acquired UDT had epididymal anomalies, vs. 46% of boys with congenital UDT who were operated on late due to parents' delay or late referral.

Kucukaydin et al. [27] and Heath et al. [13] found that testes in superficial inguinal position had fewer epididymal anomalies than had other forms of UDT. In our study, most testes in the superficial inguinal pouch were acquired UDT. Those two studies did not distinguish between congenital and acquired UDT. One might speculate, however, that they in part were acquired UDT. This would confirm our finding that epididymal anomalies mostly occur in congenital UDT.

Our data showed that epididymal anomalies were most frequent in patients with a wide open processus vaginalis. An open processus vaginalis is indeed most common in congenital UDT. All studies on the association between epididymal anomalies and the patency of the processus vaginalis report higher incidences of epididymal anomalies in patients with an open, especially wide open, processus vaginalis [5,7,9,11-13].

Finally, although surgical findings between congenital and acquired UDT unmistakably differ, it is debatable whether these two conditions represent two truly different aetiologies or rather

a spectrum of anomalies, i.e. congenital UDT, retractile testis and acquired UDT. Retractile testes have been reported to progress to acquired UDT during follow-up. In addition, reduction in germ cells have been reported not only for congenital UDT but also for retractile testes and acquired UDT [2]. Long-term studies of testicular function of these latter testes, which are not always situated in the scrotal environment, might provide more insight into the aetiological differences.

In summary, we found major differences in preoperative testis position as well as intraoperative anatomical findings between congenital and acquired UDT. First, the most caudal testis position in congenital UDT was at the inguinal region or at the scrotal entrance, whereas most of the acquired UDT could often be pushed down well below the scrotal entrance into an unstable scrotal position. Second, at surgery most congenital UDT were found inside or just outside the inguinal canal, whereas acquired UDT were mostly located in the superficial inguinal pouch. Third, congenital UDT were associated with more epididymal anomalies combined with a small open or wide open processus vaginalis in comparison with acquired UDT.

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Part II

Evaluation of traditional fertility parameters after different treatments for congenital and acquired undescended testes

Chapter 4

Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study.

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Abstract

Men with a history of congenital undescended testes (UDT) have an increased risk of fertility problems. Despite no definitive proof, current guidelines recommend early surgical intervention because this may have a positive effect on future fertility potential by preventing degenerative changes of the testes in early life. Also surgical intervention facilitates observability of the testes in view of possible malignancy. We evaluated testicular function in adult men with previous UDT treated at different ages before puberty.

A long-term follow-up study of men with previous UDT was performed. Andrological evaluation included medical history taking, physical examination, scrotal ultrasound, determination of reproductive hormones, and semen analysis. Findings were compared with those of a control group of men with normal testicular descent. The influence of age at orchiopexy on future fertility parameters was evaluated in a multivariate regression analysis.

62 men were included of whom seven had had bilateral UDT. 24 patients had had their orchiopexy before the age of 24 months of whom eight men before 12 months of age. 48 men had had unsuccessful luteinizing-hormone-releasing-hormone (LHRH) nasal spray treatment during childhood, whereas 14 of 24 men operated before 24 months of age had not received LHRH treatment before orchiopexy. Fertility potential in men with a history of UDT is compromised in comparison with controls. We could not detect any influence of age at orchiopexy on fertility parameters. However the number of patients operated before the age of 12 months is limited.

This study does not support the assumption that early orchiopexy results in better fertility potential.

Introduction

Congenital undescended testis (UDT), also known as congenital cryptorchidism, occurs in 2-9% [1] of male newborns. Spontaneous descent may occur in the first year of life, especially during the first 3 months [1]. UDT may lead to fertility problems later in life, especially in bilateral cases[2-4]. Randomized controlled studies have shown overall low success rates of hormonal therapy. Therefore, hormonal therapy is no longer recommended for the treatment of cryptorchidism [5]. The standard treatment today is a surgical one: orchiopexy. Judging from histopathological degenerative changes in the testis by the 18th month of life, early orchiopexy may prevent testicular maldevelopment and thus have a beneficial effect on fertility [6]. Current guidelines recommend that orchiopexy should be performed before the age of 18 months [7].

We report a long-term follow-up study aimed at measuring fertility potential in men treated for congenital UDT at various prepubertal ages. We aimed to answer the following: (i) What is the extent of fertility damage in men with a history of UDT in comparison with a healthy control group?, (ii) What is the influence of age at orchiopexy on the fertility parameters in adult life?, and (iii) Are there any differences in fertility parameters between men with unilateral and bilateral UDT.

Participants and methods

Participants

Subjects from a placebo-controlled study on effects of luteinizing-hormone-releasing-hormone (LHRH) nasal spray, carried out between 1982 and 1985, were assessed for eligibility [8]. Those subjects were boys aged between 1 and 12 years with unilateral or bilateral UDT who underwent orchiopexy at different ages before puberty if the UDT did not descend after LHRH nasal spray treatment.

To increase discriminative power of the present study we also assessed eligibility of men operated on before 2 years of age in our hospital between 1982 and 1990, applying the same inclusion and exclusion criteria as for the LHRH-study [8]. A total of 225 men, with 252 congenital UDT, could be contacted for the present study.

The presence of retractile testes had been excluded under general anaesthesia. Surgical findings were available for all men, classified as published previously [9]. In short:

Intraoperative location: (i) Absent; (ii) Intraabdominal; (iii) Inguinal canal; (iv) External annulus; (v) Superficial inguinal pouch.

Processus vaginalis: (i) Closed; (ii) Small open; (iii) Wide open.

Methods

All 225 men were sent an invitation by post, including the request to return the enclosed response card. A reminder was sent to those who did not return the response card. Additional information about the study was sent to men who considered participating in the study. Again, eligible candidates who did not respond were contacted by post. Those who declined participation were asked for their reasons and were invited to fill in a questionnaire regarding fertility and risk factors for subfertility or infertility other than UDT. Participation involved a single hospital visit including medical history taking, physical examination, scrotal ultrasound, venous puncture, and semen sample delivery.

History

We focused on risk factors for fertility problems other than cryptorchidism. Special attention was paid to attempting fatherhood, duration of child-wish (months), and time to first pregnancy (TTP) (months).

Physical examination and scrotal ultrasound

Two investigators (including first author) conducted a physical examination and performed a scrotal ultrasound to measure testicular volume and to check for the presence of a varicocele in a standardized procedure using a high-resolution duplex ultrasound machine (Toshiba Nemio model 17 SSA-550A; Toshiba Medical Systems Corporation, Otawara-shi, Tochigi, Japan) with a transducer frequency of 7.5-12 MHz.. A testicular volume of 15 ml or greater was considered normal.

Blood analysis

A venous blood sample was taken, preferably before 10:00 AM and before 11:00 AM at the latest. Serum samples were assayed for luteinizing hormone (LH, reference value 1.5-8 IU/L) and follicle stimulating hormone (FSH, reference value 2-7 IU/L) using fluorescence-based immunometric methods (Immulite 2000; Siemens-DPC, Los Angeles, CA, USA), testosterone (reference value 10-30 nmol/L) using a coated tube radioimmunoassay (Siemens-DPC), and

inhibin B (InhB, reference value ≥ 150 ng/L) using an enzyme-immunometric method (Oxford BioInnovation, Oxford, UK).

Semen analysis

A semen sample produced by masturbation after a 3-5 day ejaculatory abstinence was analysed according to the recommendations from the most recent WHO-manual [10]. Within one hour, after liquefaction, semen volume (reference value ≥1.5 ml), sperm concentration (reference value ≥15 10*6/ml), total sperm count (reference value ≥39 10*6/ ejaculate), progressive motility (reference value ≥32%), and morphology (reference value ≥4% normal forms) were determined.

Control group

Fifty-three healthy men without a history of UDT served as a control group. These men took part in a study investigating fatherhood in tall men (n=153) after the use or non-use of sex steroid treatment in adolescence to reduce final height [11]. These men had the same evaluation as the study participants, except for morphological evaluation of semen.

In that study men with endocrine or metabolic disorders were excluded. As a control group we used the 56 men who did not receive sex steroids, of whom we excluded 3 men referable to a history of orchiopexy or treatment with radio- or chemotherapy [11].

Statistical analysis

Continuous variables were tested with the Mann-Whitney U test; categorical variables with Fisher's exact test. First, characteristics of participants were compared with those of non-participants. Second, participants' information regarding medical history and paternity was compared with that of non-participants who returned the questionnaire.

The Kaplan-Meier survival method served to estimate the probability of conception in participants and controls; the Wilcoxon (Breslow) test served to test if the participants' average conception rate differed from that in controls. Furthermore, fertility parameters (testicular volume, endocrinological values, and semen parameters) were compared between the two groups. In case of bilateral UDT, testicular volume was taken to be the mean of the volumes of the two testes.

Age at surgery was analysed as a continuous variable in several multivariate logistic regression analyses performed to establish relationships between fertility parameters (testicular volume, endocrinological results, and all semen parameters) and age at surgery. Fertility parameters were analysed by means of the chance of being below or above the reference value. Known confounders such as smoking, varicocele, and Body Mass Index (BMI) were corrected for in these analyses [12]. Other independent variables were uni-/bilateral UDT, intraoperative testis position, and patency of processus vaginalis. For all analyses a two-sided *p*-value of less than 0.05 was considered statistically significant.

Ethics

The study was reviewed and approved by the Medical Ethical Review Board at Erasmus University Medical Center in Rotterdam (MEC number 2004-206). Written informed consent was obtained from all men who agreed to participate in the study.

Results

Participants

Sixty-two out of the 225 eligible men (28%) participated (Fig. 1). Non-participants stated time constraints and bothersomeness of the investigations as main reasons for refusal. Age at orchiopexy, present age, and distribution of unilateral and bilateral UDT did not significantly differ between participants and non-participants (Table 1). The participation rate of men operated at younger age was not statistically different from that of men operated at older age when the cut-off was set at 12, 18 or 24 months of age at orchiopexy (age ≤12 months p-value 0.248; age ≤18 months p-value 0.176; and age ≤24 months p-value 0.652).

Thirty-eight men who responded but did not participate returned the questionnaire. Present age and age at orchiopexy did not significantly differ between participants and men who returned the questionnaire without further participation. Proportions of men attempting fatherhood, number of pregnancies, TTP, and proportions of men having an active child-wish at time of recruitment did not differ between the participants and non-participants returning the questionnaire. In neither group TTP exceeded 12 months. The duration of child-wish was significantly longer in participants than in non-participants who had returned the questionnaire [median (range); 12 (2-36) vs. 2 (0-5) months respectively; p-value 0.03].

Comparison of fertility parameters; participants with a history of unilateral UDT vs. healthy controls (Table 2)

Physical examination and scrotal ultrasound:

Participants' volume of the previously UDT, measured by ultrasound, was significantly smaller than the controls' mean testicular volume. Moreover, the volume of the normally descended contralateral testis was also significantly smaller compared with the control group. All men in the control group had a normal testicular consistency; 29 of 55 participants (53%) had uni- or bilateral soft testicular consistency (p-value <0.001). Varicocele occurred more often in the control group, but the difference was not statistically significant.

Endocrine function:

Endocrinological data were obtained from 54 participants; one participant refused blood sampling. Both Leydig cell function represented by LH and testosterone as well as Sertoli cell function represented by FSH and InhB did not differ between the control group and the participants.

Semen analysis:

Five men in the control group had undergone vasectomy and therefore semen analysis was not performed. Participants had a significantly lower median concentration, more often oligozoospermia according to the WHO-reference values[10], and less progressive motile sperm than the control group. None of the participants was diagnosed with azoospermia.

Paternity:

A higher proportion of men in the control group attempted fatherhood. More men in the control group succeeded in comparison with the participants, however this difference was not statistically significant (participants: 6 of 11 (55%) vs. controls: 25 of 29 (86%); p-value 0.08). Based on low numbers of men who attempted fatherhood, TTP and duration of child-wish were not statistically significantly different between these two groups (data not shown). The result of the Breslow test, which took time to conception into account was that the control

group had a higher probability of conceiving, however, this was not statistically significant (0.07 pregnancy/month for unilateral group vs. 0.12 pregnancy/month for controls; p-value 0.28) (Fig. 2).

Influence of age at orchiopexy on fertility parameters

The participants' median age at orchiopexy was 3.0 years (range 0.1 - 14.6 years). Eight boys had been operated on before the age of 1 year, 12 before 18 months of age, and 24 before the age of 2 years.

Physical examination and scrotal ultrasound:

In the complete group as well as in the unilateral group only, testicular volume of the previous UDT, testicular volume of the contralateral normally descended testis, testicular consistency, and incidence of varicocele did not statistically differ when cut-off values for age at surgery were used (12 months, 18 months, and 24 months at operation).

Endocrine function:

Both in the complete group of participants and the unilateral group only, hormone levels except LH did not differ at the cut-off values of 12 months, 18 months or 24 months at orchiopexy. LH levels were significantly lower in the group operated at older age when applying cut-off values for age at orchiopexy (12 months, 18 months, and 24 months at operation) (Mann-Whitney U test).

Semen analysis:

Both in the complete group of participants and in the unilateral group only, sperm parameters did not differ at the cut-off values of 12 months, 18 months, and 24 months at orchiopexy.

Multiple regression analyses:

Figure 3 shows scatter plots of the relations of age at orchiopexy with testicular volume, FSH, InhB, and sperm concentration, respectively. We could not detect any influence of age at orchiopexy on these fertility parameters.

Table 3 presents the results of the multiple regression analyses for age at orchiopexy as a

continuous variable and its effect on testicular volume, hormonal values, and semen parameters.

Age at orchiopexy had no significant influence on fertility parameters, after correction for known confounders and other independent variables (smoking, varicocele, BMI, uni-/bilateral UDT, intraoperative testis position, and patency of processus vaginalis).

Influence of bilateral UDT on fertility parameters; comparisons with controls and unilateral UDT

Seven men of 62 had a history of bilateral UDT, two men had had an orchiopexy before the age of two and five men were operated after 2 years of age.

Physical examination and scrotal ultrasound:

The mean testicular volume in the group of men with bilateral UDT was statistically smaller in comparison with the control group [median (range): 7.6 ml (6.2 – 18.1) vs. 15.8 ml (8.1 – 27.0) respectively; *p*-value 0.01]. Five of seven men (71%) had uni- or bilateral soft testicular consistency compared with none of the men in the control group (*p*-value <0.001). No differences in physical examination or scrotal ultra sound results were found when comparing the bilateral group with the unilateral group.

Endocrine function:

In comparison with the control group, the bilateral group showed values, which indicate worse Sertoli cell function represented by both FSH and InhB. The median FSH-value was 8.2 IU/L (range 1.6 – 28.8) for the bilateral UDT group and 4.5 IU/L (range 1.6 – 13.9) for the control group (*p*-value 0.05). The median InhB value in the bilateral group was significantly lower compared with that in the control group: median (range) 125.0 ng/L (1.0 – 364.0) vs. 168.0 ng/L (96.0 – 488.0) respectively (*p*-value 0.02).

When comparing participants with unilateral UDT with participants with bilateral UDT no statistically significant differences in endocrine function were found. Borderline significant values were found for FSH and InhB. FSH was higher in the bilateral group [median (range) 8.2 IU/L (1.6 – 28) vs. 4.8 IU/L(0.7 – 21.2); *p*-value 0.08). InhB was lower in the bilateral group [median (range)125 ng/L (1.0 – 364.0 vs. 157.0 ng/L (22.0 – 554.0); *p*-value 0.09].

Semen analysis:

In comparison with the control group, men with previously bilateral UDT had significantly lower sperm concentration [median (range): 0.6 x10*6/ml (0.1 – 364.0) vs. 54.5 x10*6/ml (0.1 – 213.0); *p*-value <0.001] and motility [median (range): 36% (1 - 60) vs. 52% (9 – 91); *p*-value 0.02).

Sperm concentration in men with bilateral UDT was significantly lower than that in men with unilateral UDT [median (range): 0.6 x10*6/ml (0.1 – 46.0) vs. 21.0 x10*6/ml (0.1 – 276.0); *p*-value 0.03]. When the cut-off value for oligozoospermia stated in the WHO 2010 guideline was used, no significant difference was found between men with unilateral and those with bilateral UDT.

Fertility parameters in participants operated before 24 months of age; comparison of men receiving LHRH nasal spray before orchiopexy vs. men not receiving LHRH nasal spray (Table 4)

Of the 62 men included in this study 24 men had had the orchiopexy before the age two years. 14 men of 24 did not receive LHRH nasal spray prior to the orchiopexy. These men were significantly younger at orchiopexy compared with the men who had received LHRH nasal spray.

Physical examination and scrotal ultrasound:

Testicular volume of the previously cryptorchid testis was significantly smaller in the group treated with LHRH nasal spray prior to orchiopexy compared with the testis volume of the men not treated with LHRH nasal spray. No difference was found when comparing testicular volume of the normally descended testis between the group treated with LHRH nasal spray and men not treated with hormonal therapy. The volume of the previously UDT of men not

treated with hormonal therapy did not statistically differ between men operated before or after 12 months of age: [median (range)) 13.1 ml (6.6 – 18.8) and 15.9 ml (12.7 – 22.9); *p*-value 0.25].

Endocrine function:

No differences could be observed in endocrine function between men who were and were not treated with LHRH nasal spray prior to orchiopexy.

Semen analysis:

No significant differences were found in semen parameters when comparing men who had had LHRH nasal spray before surgery and men who were only treated with orchiopexy.

Discussion

The most important finding of this study is the lack of an age-dependent effect of orchiopexy on fertility parameters in men with previous congenital UDT.

Fertility potential can be evaluated using semen analysis. In this study both semen concentration and motility were significantly lower in participants than that in the control group. Cortes et al. published data on semen parameters in patients with unilateral and bilateral UDT. In comparison with our study, the sperm parameters found in their studies were better [3,4]. However, in those studies the age at surgery was relatively high, which might indicate a higher proportion of men having acquired UDT instead of congenital UDT. Also it is not clear whether retractile testes have been excluded in those studies. Many studies have been published on semen parameters in patients with UDT. Such studies are not always easy to compare because of several reasons; (i) different therapeutic strategies, (ii) variation in reporting the results (mean values, median values or percentage normal/abnormal), (iii) the WHO-reference values for semen analysis have changed over time, (iv) uncertainty whether groups contain only congenital UDT or also acquired UDT, (v) groups may contain unilateral or bilateral UDT or a combination of both, (vi) unclear about the exclusion of retractile testis[3,4,13-19]. In this study, men with retractile testis or acquired UDT were excluded this might be an explanation for our low sperm concentration in comparison with the literature.

Although some studies have suggested that orchiopexy at younger age results in better semen parameters [13,15,18] results of this study show no favourable effect of early treatment on semen characteristics, in line with other studies [14,16,17,19]. No study which evaluates age at surgery as a continuous variable finds statistically significant correlations with semen parameters [14,16,17,19]. Two studies found an effect of age at surgery for patients with bilateral UDT, but not for unilateral UDT [15,18]. Canavese et al., in a study on orchiopexy within the first 2 years of life found that age at surgery was inversely related to sperm concentration and motility[13].

Different results on the effect of hormonal treatment on fertility potential or germ cell development have been reported. In the study by Canavese, men operated at a younger age had significantly more often received hormonal therapy (LHRH nasal spray and subsequently human chorionic gonadotropin i.m.) prior to surgery [13]. In a study by Hadziselimovic et al., in which the effect of LHRH nasal spray on testicular descent was analysed, neither a stimulatory effect nor an inhibitory effect on the number of germ cells was seen [20]. This in contrast to Cortes et al., who found a negative effect on germ cells in 1 to 3-year-old boys with cryptorchidism after gonadotropin releasing hormone (GnRH) [21]. They hypothesized that these young boys may be sensitive for exogenous GnRH stimulation resulting in increased FSH, LH, and testosterone, which may have a negative impact on germ cells. In this study, all participants who were operated after 24 months of age received LHRH nasal spray prior to orchiopexy, although 10 of the 24 men who were operated before 24 months received this hormonal treatment and 14 men did not. We hypothesize that our LHRH treatment, at younger age, is very unlikely to have influenced later fertility because at the time of treatment no evidence of stimulation of the hypothalamo-pituitary-gonadal axis was found [8]. At adult age only testicular volume of the previously undescended testis appeared to be smaller in the group treated with LHRH nasal spray prior to surgery compared with men treated with only orchiopexy. Thereby testis volume of the contralateral testis in case of unilateral UDT did not differ between men with or without hormonal therapy. Endocrinological values and semen analysis did not show statistical differences between participants receiving hormonal therapy and those not receiving hormonal therapy below the age of 24 months.

Testicular volume is an accepted predictor for spermatogenesis [22]. In this study, participants' testicular volume was significantly lower than that of the control group. However, age at orchiopexy did not influence testicular volume in adulthood. As stated above, the group of men of this study who had not received hormonal therapy had larger testicular volumes. When evaluating the age effect in this group also no effect of age at orchiopexie on adult testicular volume could be found. Other studies on testicular volume and age at orchiopexy imply that younger age at surgery might result in higher testicular volume [15,23,24]. To our knowledge only one randomized prospective study showed a beneficial effect of early orchiopexy on testis volume. At 4 years of age testis volume was larger in patients operated at 9 months compared

with patients operated at the age of 3 years [25]. However, the follow-up period differed between the two groups and the question remains whether adult testicular volume would still be different between the two groups and if so, what the effect would be on fertility.

Other indicators of spermatogenesis are FSH and InhB levels. UDT have been related to lower InhB and higher FSH levels [2]. In this study, FSH levels and InhB levels did not significantly differ between participants and the control group. Age at orchiopexy was not significantly correlated with FSH and InhB levels. This in contrast to a study by Coughlin et al., in which age at surgery negatively correlated with InhB and positively with FSH levels [14]. However, in that study a multivariate analysis correcting for known confounders was not performed. Some histological studies not only suggest maldevelopment of germ cells but also of Leydig cells [26]. Hormonal evidence for Leydig cell dysfunction was found in adult patients after a late orchiopexy [27]. In this study, age at orchiopexy had no statistically significant influence on Leydig cell function.

Men with a history of bilateral UDT face more fertility problems than do men with previously unilateral UDT [16,18]. In this study sperm concentration was significantly lower in patients with bilateral UDT (Mann-Whitney U test). Multivariate analyses, however, showed that having uni- or bilateral UDT did not predict the chance of having a sperm concentration below or above 15 x 10*6/ml. The analysis might be flawed, however, by low number of men with bilateral UDT (N=7).

Another way to evaluate fertility is looking at paternity. Lee et al. reported paternity proportions of 89.7%, 65.3%, and 93.2% in, respectively, unilateral UDT, bilateral UDT, and healthy controls [2]. Likewise, in this study, paternity in the participants was borderline significantly lower than in the control group. Lee et al. found that age at orchiopexy was not correlated with paternity [28]. Due to the small numbers of men who fathered children or attempted fatherhood in our study we cannot confirm this result.

Early operation is hypothesized to have a favourable effect on germ cell development. In the first year of life, neonatal germ cells transform into spermatogonia. This process is important for future fertility because spermatogonia are the progenitor cells for future spermatogenesis. In boys with UDT, the process of germ cell maturation is impaired [26]. Germ cell numbers start to deviate as from 18 months old [6]. Based on these observations the recommended age for orchiopexy was gradually lowered to under the age of 12 months [6]. The Nordic consensus recommends surgery between 6 to 12 months of age, but not earlier given the possibility of spontaneous descent during the first months of life [5]. The question remains why early operation, which might prevent histological changes, had no beneficial effect on fertility parameters in this study. As only eight patients were operated on or before the age of 12 months, a definite conclusion cannot be drawn. Our results suggest a congenital malfunction

of the testis that cannot be influenced by reducing duration of the non-scrotal position. Huff et al. found similar, but less severe histological changes in the contralateral normally descended testis [29]. Also Zivkovic found significantly lower numbers of germ cells per tubule in contralateral normally descended testes in patients with unilateral UDT in comparison with spontaneously descended testes in healthy controls [30]. Indeed, both testes might be affected in case of unilateral UDT, for in our study the volume of the contralateral normally descended testis was also significantly smaller than that of controls.

A limitation of our study is the fairly low inclusion rate (28%). The inclusion rate in other studies varied between 29 and 73%[3,4,13,15,16,18]. Three studies had a higher number of participants than our study [3,14,16]. A possible explanation for our low inclusion rate is the long interval between operation and inclusion. Thereby, in our study patients had no regular check-ups after operation. Patients might be more motivated to participate when the treatment is more recent or when follow-up is more frequent. In this study age at orchiopexy, uni- or bilateral cryptorchidism, and current age did not differ between men who did not respond and men who responded by questionnaire or participated. This suggests that the group of men who completed follow-up is a representative part of the whole group. The number of men having an active child-wish did not differ between the men who responded by questionnaire and the men participating. Selection bias may have occurred, however, because the participants' duration of child-wish was significantly longer than that of those who only returned the questionnaire. The duration of child-wish is not to be mistaken for actual clinical infertility. The questionnaire was not validated to produce such information. Selection bias is always an issue in fertility studies, because men with fertility problems might be more motivated than fertile men to participate. We presume that the risk for selection bias because of fertility problems is the same in both the participants and the control group (the participation rate in the control group was 31%) as the control group was not a group of chart-selected, mostly fertile men. A high occurrence of varicocele in tall men is described [31]. In this study the incidence of varicocele in the control group was twice as high compared with that in the patient group. In our multivariate regression analyses we corrected for varicoceles.

In conclusion, the present long term follow-up study could not find a relation between the age at orchiopexy and fertility parameters for patients with a history of unilateral UDT, most of them after unsuccessful LHRH treatment. As only eight patients had had their orchiopexy in the first year of life, this conclusion has to be taken with caution for patients operated before 12 months of age. This conclusion might also not be legitimate for bilateral UDT because no more than seven men with bilateral UDT participated. As congenital UDT are not expected to descend spontaneously after the first year of life, orchiopexy is needed for psychosocial reasons, prevention of torsio, and better observability of the testes in view of possible malignancy in young adulthood.

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Chapter 5

Fertility potential in a cohort of 65 men with previously acquired undescended testes

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Abstract

Purpose:

To evaluate testicular function in men with previously acquired undescended testes (AUDT) in whom spontaneous descent was awaited until puberty followed by orchiopexy in case of nondescent.

Methods:

Andrological evaluation including paternity, scrotal ultrasound, reproductive hormones, and semen analysis was performed in three groups: men with AUDT, healthy controls, and men with previously congenital undescended testes (CUDT).

Results:

In comparison with controls, men with AUDT more often had significantly abnormal testicular consistency, smaller testes, lower sperm concentration, and less motile sperm.

Except for more often a normal testicular consistency in men with AUDT, no differences were found between men with AUDT and CUDT.

Also, no differences were found between men with AUDT which had spontaneously descended and men who underwent orchiopexy.

Conclusions:

Fertility potential in men with AUDT is compromised in comparison with healthy controls, but comparable with men with CUDT. This suggests that congenital and acquired UDT share the same aetiology.

No significant difference could be found between men who had spontaneous descent and men needing orchiopexy. However fertility potential is unknown for men after immediate surgery at diagnosis, and this should be subject for future studies.

Introduction

Undescended testis (UDT) is a frequent urogenital abnormality in boys with an incidence around 1% at one year of age [1]. The abnormality may be of congenital or acquired origin [2,3]. In the congenital type a stable scrotal position was never reached. Acquired UDT is defined as a palpable UDT that previously had a normal scrotal position, as documented by a physician. Acquired UDT is seen in 1-3% of boys during childhood [4,5] and its aetiology, natural course, and treatment are still debated. Two studies on the proportion of (pre) pubertal spontaneous descent in boys with acquired UDT found a prevalence of 57% and 71.4% spontaneous descended testes [6,7]. Congenital UDT is associated with infertility [8,9]. However, little is known about fertility in men with previously acquired UDT (AUDT) and the effect of a "wait-and-see until puberty" policy [6]. The aim of our study was to evaluate the impact of AUDT on fertility parameters in comparison with controls and men with previously congenital UDT (CUDT). Furthermore, we evaluated testicular function in men with AUDT after spontaneous descent or orchiopexy.

Materials and Methods

Acquired UDT group

Between 1982 and 2004, boys with acquired UDT referred to Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, and the Medical Center, Alkmaar, were followed by three experienced physicians (S.de.M.K.-S., F.W.H., or W.H.) annually until puberty. Acquired UDT implied that a previous normal stable scrotal position was documented at least once either by the Regional Youth Health Care Institution, general practitioner, or hospital. Furthermore, the testis had to be palpable without ipsilateral, inguinal, clinically present pathological findings or previous ipsilateral, inguinal surgery, and the most caudal testicular position after manipulation had to be nonscrotal or unstable scrotal position. Spontaneous descent was awaited until at least Tanner stage P2G2, after which orchiopexy was performed in case of nondescent. Findings at physical examination were registered on a standardized form. Retractile testes were excluded. Orchiopexy was performed by paediatric surgeons with years of experience performing orchiopexies.

In 2009, a total of 231 men with 287 AUDT were screened for eligibility for this study, of whom 99 men had undergone orchiopexy. Seven men were excluded mostly because of chromosomal abnormalities and dysmorphic syndromes besides UDT. For 17 no current address was available, thus 207 were invited to participate.

Control group and congenital UDT group

Fifty-three healthy men without a history of UDT served as controls. These men previously participated in a study evaluating fatherhood after use or non-use of sex steroids treatment during adolescence to reduce final height. Men, who were not treated, were used as our control group. Furthermore, we created a CUDT group (N=62, 69 testes) of men who participated in a study evaluating fertility potential after treatment of congenital UDT during childhood. Both groups were previously described in detail [10,11]. These men had the same evaluation as men with AUDT, except for morphological evaluation of semen, which was not performed in the controls.

Methods

The methods for recruitment and andrological evaluation were published previously [11]. In short, all 207 men were invited to participate by post. Those who refused were invited to fill out a questionnaire on fertility and risk factors for subfertility or infertility. Participation involved a single hospital visit including medical history taking, physical examination, scrotal ultrasound, venous puncture, and semen sample delivery. Special attention was paid to attempting fatherhood, duration of child-wish, and the time to first pregnancy (TTP).

Testicular volume and presence of testicular and epididymal abnormalities and varicocele were assessed by physical examination and confirmed by scrotal ultrasound.

The following endocrinological values were evaluated: luteinizing hormone (LH) and follicle stimulating hormone (FSH), testosterone, and inhibin B.

Semen parameters that were evaluated were analysed according to the WHO manual of 2010 [12], which included volume, concentration, total sperm count, progressive motility, and morphology.

Statistical analysis

Continuous variables were tested with the Mann-Whitney U test; categorical variables with Fisher's exact test. The Kaplan-Meier survival method was used for time-to-event analyses, Wilcoxon's test was used to test between group differences in such cases. Multivariate logistic

regression analyses were used to test the relationship between bivariate categories and their predictors. For example, we have studied the relationship between fertility parameters (bivariate classes: being below or above the reference value) and the predictor "type of UDT" controlling for known confounders: smoking, varicocele, and Body Mass Index (BMI) and unilateral-bilateral UDT. A two-sided *p*-value of less than 0.05 was considered statistically significant.

Ethics

The study was reviewed and approved by the Medical Ethical Review Committee at Erasmus University Medical Center in Rotterdam (MEC number 2004-206). Written informed consent was obtained from all participants.

Results

Inclusion of men with AUDT

Sixty-five (31%) out of the 207 eligible men participated (Fig. 1). Most of the 49 nonparticipants who nevertheless returned the questionnaire (35%) mentioned time constraints and bothersomeness of the investigations as reasons for refusal. Only two men reported that having fathered children was also a reason to decline participation. Table 1 shows the characteristics of study participants and nonparticipants. No significant differences were found between participants and men returning the questionnaire in terms of the proportion of men attempting fatherhood, number of pregnancies, TTP, and proportions of men having an active child-wish. One nonparticipant with bilateral acquired UDT with spontaneous descent replied that he had azoospermia.

Fifty out of the 65 men with AUDT had unilateral AUDT. Spontaneous descent had occurred in 32 participants (24 unilateral and 8 bilateral). Median age at orchiopexy (N=33; 26 unilateral and 7 bilateral) was 13.2 years (range 4.75 to 17.8 years). Most important criteria for prepubertal orchiopexy were inguinal complaints or ipsilateral inguinal hernia.

Comparisons of fertility parameters

Men with unilateral AUDT vs. controls (Table 2)

Testicular volume, both of the previously undescended one and the contralateral testis was significantly smaller than that of controls. Testicular consistency was normal in all controls; 13 men with AUDT (26%) had unilateral or bilateral soft testicular consistency (*p*-value < 0.001).

Endocrinological data were obtained from 49 men with AUDT: 1 of them refused blood sampling. Endocrine function did not differ significantly between men with AUDT and controls.

One man with AUDT was unable to produce semen. Semen of five controls who had undergone vasectomy was not analysed. Concentration and progressive motility in men with AUDT were significantly lower than those in controls. Azoospermia was noted in four men with AUDT (three spontaneous descent and one orchiopexy).

Controls, who were significantly older than men with AUDT, significantly more often attempted fatherhood. However fatherhood, TTP, cumulative probability of conception, and duration of child-wish did not statistically significantly differ between men with AUDT and controls.

Two partners of controls had undergone intra uterine insemination. The reason was unknown for one couple and for the other couple it was caused by a female problem. The partner of one man with AUDT who had had orchiopexy, needed assisted reproductive treatment (ART) because she had a fertility problem.

Men with unilateral AUDT vs. men with unilateral CUDT (Table 2)

Normal testicular consistency was significantly more often found in men with AUDT than in men with CUDT (74% vs. 53%; *p*-value = 0.03).

Endocrine function and semen analysis did not differ between the AUDT group and the CUDT group.

Paternity did not differ between men with AUDT and men with CUDT. As stated above, one couple, with a man with AUDT, needed ART because of a female factor fertility problem. None of the unilateral CUDT attempting fatherhood needed ART.

Men with bilateral AUDT vs. controls vs. men with bilateral CUDT (Table 3)

Testicular consistency was normal in 9 of 15 men with bilateral AUDT, while it was normal in all controls (p-value < 0.001). Mean testicular volume of men with bilateral AUDT was significantly

smaller than that of controls [median (range) 10.9 (5.2 – 14.6) vs. 15.8 (8.1 – 27.0); *p*-value < 0.001]. Testicular volume did not differ between the AUDT group and the CUDT group.

Men with bilateral AUDT had significantly higher LH and FSH, and lower inhibin B, semen concentration and motility compared with controls. Azoospermia was noted in one man with bilateral AUDT; both testes had descended spontaneously.

Endocrine function and semen analysis did not differ between the AUDT group and the CUDT group.

In the group of men with bilateral AUDT (N=15) only 1 (25%) of 4 men attempting fatherhood succeeded vs. 25 (86%) of the 29 controls (*p*-value = 0.02).

Men with bilateral CUDT significantly more often attempted fatherhood than men with bilateral AUDT, but success rates did not differ. Two couples with men with bilateral AUDT who had had orchiopexy needed ART. One couple had male fertility problems and for the other couple a female factor was identified.

Men with unilateral AUDT vs. men with bilateral AUDT (Table 4)

When evaluating unilateral AUDT with bilateral AUDT only significant differences were found in comparing semen analysis. Unilateral AUDT had a higher semen concentration [median (range) 20.0 (0 – 205.0) vs. 8.2 (0 – 60) *p*-value = 0.04] and higher percentage of motile sperm [median (range) 42.0 (3 – 75) vs. 25.0 (0 – 61); *p*-value = 0.06] and less often oligozoospermia [number (percentage) 19 (39) vs. 10 (71); *p*-value = 0.04] and astenospermia [number (percentage) 10 (22) vs. 8 (62); *p*-value = 0.01] in comparison with bilateral AUDT. All other variables did not show statistically significant differences.

Men with AUDT: spontaneous descent vs. orchiopexy (Table 5)

Testicular volume, testicular volume of contralateral healthy testis in case of unilateral AUDT, testicular consistency, reproductive hormones, and semen analysis did not significantly differ between men with AUDT whose UDT descended spontaneously and those who underwent orchiopexy. This was the same for the evaluation of the complete group of men with AUDT, men with unilateral AUDT, and men with bilateral AUDT.

In the orchiopexy group more attempts at fatherhood were noted, also when evaluating only men with unilateral AUDT; however fatherhood, TTP, and duration of child-wish did not differ.

Multivariate logistic regression analyses

Men with AUDT vs. men with CUDT

Type of UDT had no significant influence on any of the fertility parameters except testicular volume [Odds ratio (OR) 0.24, 95% confidence interval (CI) 0.08 – 0.70; *p*-value = 0.009]. Men with AUDT had a lower chance of having a volume equal or greater than 15 ml. Having bilateral UDT was associated with oligozoospermia and astenospermia [OR 0.23, 95% CI 0.08 – 0.64; *p*-value = 0.005 and OR 0.32, 95% CI 0.11 – 0.88; *p*-value = 0.03, respectively].

Men with AUDT: spontaneous descent vs. orchiopexy

No significant differences in fertility parameters were found between men with a spontaneous descent and men with orchiopexy. Having bilateral UDT decreased the chance of having a normal sperm concentration and motility [OR 0.21, 95% CI 0.06 – 0.80; *p*-value = 0.02 and OR 0.15, 95% CI 0.04 – 0.67; *p*-value = 0.01]. Age at orchiopexy had no influence on fertility parameters (data not shown).

Discussion

To our knowledge, this is the first study regarding fertility parameters in men with previously acquired UDT. Fertility parameters in these men were poorer than those in controls but did not differ from those in men with previously congenital UDT. Finally, fertility potential did not significantly differ between men with AUDT in whom spontaneous descent was successfully awaited and those who underwent orchiopexy.

The timing of orchiopexy in acquired UDT is debated [(3)]. Some studies advise immediate surgical intervention because of significant risk of developing degenerative histological changes, comparable to histological changes found in congenital UDT [(13)]. A consensus meeting in 2006 recommended immediate surgery despite the fact that many of these testes will descend spontaneously. It was argued that spermatogenic and endocrine function and not anatomical position should be the primary aim for treatment [(14)]. So far, no evidence is available that fertility improves with immediate surgery. There may be fewer fertility problems, however, since the crucial development of type A spermatogonia has probably

already occurred during the first year of life before the testis ascended [15]. This, however, remains to be proven.

Fertility can be evaluated by semen analysis. Unfortunately, evaluating fertility with semen analyses has some shortcomings. Biological variation of consecutive semen samples in an individual can be substantial [16]. Also, semen analyses have large inter- and intralaboratory variability. In our study both concentration and motility in the AUDT group were lower than those in controls. Thereby, we found higher semen concentration and higher percentage of progressive motile sperm in men with unilateral AUDT in comparison with bilateral AUDT. This is in line with other studies that show better semen parameters in men with unilateral UDT [17,18]. Ong et al. suggested that in ascended testes subfertility is caused by heat-induced defective spermatogenesis [19], whereas Rusnack et al. hypothesized that an endocrine defect can diminish the number of germ cells [13]. In both studies germ cell count in acquired and congenital UDT was comparable. Moreover, the total and differential germ cell counts were similarly diminished for the contralateral normally descended testis in acquired as well as congenital unilateral UDT compared with controls. Therefore, Rusnack et al. argued against a heat-induced defect since no thermal effect can influence the normally descended testis. This is in line with our previous study, in which sperm quality after orchiopexy is independent of age at surgery and thus independent of duration of possible thermal damage [11]. The present study, also could not detect an age-specific influence on fertility potential. Some studies, not distinguishing between acquired and congenital UDT, showed a beneficial effect of early orchiopexy [18,20,21] on sperm quality, while others did not find such a correlation [17,22-24]. It is difficult to draw any conclusion from these studies for acquired UDT since previous testicular position is not documented.

Approximately one-third of retractile testes later become acquired UDT [2,5]. A study by Han et al. showed histopathological changes in 40% of biopsied retractile testes [25]. Another study found a mean decreased tubular fertility index value in men with acquired UDT, implying impaired spermatogenesis similar to that in congenital UDT [26]. Tanyel hypothesized that congenital and acquired UDT share the same aetiology [27]. Our findings seem to confirm this hypothesis, seeing that there were no significant differences in semen parameters between men with AUDT and men with CUDT. Therefore, in our view UDT could be a spectrum of anomalies encompassing congenital UDT, retractile testes, and acquired UDT. This theory is also suggested by Hack et al. [28]. They speculate that acquired UDT are actually previously missed congenital UDT. These testes are in scrotal position at birth in spite of a short funiculus. However, the testis ascends while the boy grows owing to the inadequate length of the funiculus.

Testicular volume is an accepted predictor for spermatogenesis [29]. In a study by Meij-de Vries et al. the volume of the AUDT after prepubertal orchiopexy measured by ultrasound was

smaller than that of the contralateral healthy testis and of the normative volume described in the literature [30]. In our study testicular volume of AUDT was significantly lower than that of controls, like in our previous study comparing CUDT with controls [11]. Univariate analysis revealed no significant difference between testicular volume in the AUDT group and that in the CUDT group. In the multivariate analysis, however, men with AUDT had a significantly higher chance than men with CUDT of having a testicular volume below 15 ml. Note that a cut-off value of 14 ml would have resulted in no difference. Neither spontaneous decent nor orchiopexy or age at orchiopexy influenced later testicular volume. Meij-de Vries et al. reported a similar finding regarding age at orchiopexy [30].
Reproductive hormones are indicators of testicular function and represent both testes. In our univariate analysis we did not find significant differences in reproductive hormones between unilateral and bilateral AUDT. We corrected for unilateral and bilateral UDT in our multivariate analyses. FSH and inhibin B are indicators of spermatogenesis, and levels were found to be higher and lower, respectively, in a previous study in patients with UDT [31]. In our study, this only held true for men with bilateral AUDT in comparison with controls. Also higher LH levels were found in bilateral AUDT compared with controls. Lee and Coughlin found no significant difference in Leydig cell function between men with UDT and controls [32].

Another method to evaluate fertility is looking at paternity. Lee et al. found a decreased paternity rate in men with previous bilateral UDT compared with unilateral UDT and controls [31]. Information on paternity rates in acquired UDT has not been reported. In our study, controls significantly more often attempted fatherhood; this could be explained by the significant age difference between controls and men with AUDT. In our study relatively few men with AUDT attempted fatherhood. Success rates only significantly statistically differed between men with bilateral AUDT and controls: significantly more controls succeeded in becoming a father. The lack of significant differences in successful attempts to fatherhood between unilateral AUDT, unilateral CUDT, and controls could be caused by a power problem. Using paternity to evaluate fertility has some shortcomings; first female fertility should be taken into account; second, men with compromised fertility with a fertile partner might still be able to father children; and third, the man presuming to be the father may not be the biological father.

This study has several limitations. First, it is uncertain whether the participants are representative for the whole group. Although, characteristics did not differ. Second, our control group might not be an optimal control group since these men are participants of a tall stature study, albeit without hormonal treatment. However, the inclusion rate for both our study as well as the tall stature study was 31%; therefore selection bias because of fertility problems seems unlikely. Also, tall height is associated with varicoceles [33]. Controls had almost twice as much varicoceles compared with participants. However this was not

statistically significant and we corrected for varicoceles in our multivariate analyses. Third, this study cannot answer the question what the fertility potential would have been if all patients were operated on immediately after diagnosis. Fourth, we tested only one sperm sample for each man and therefore cannot account for biological variation of semen quality in these men. An article by Christman et al., however, found that a single measurement might be appropriate to classify fertility problems in young men with a history of cryptorchidism [34].

In conclusion fertility in men with AUDT is compromised in comparison with men with normally descended testes but comparable with men with CUDT. These results suggest that acquired UDT and congenital UDT share the same aetiology. Acquired UDT merely represent a part of a spectrum of UDT and is not a variant of normally descended testes. Moreover, no significant differences could be found between men who had AUDT which descended spontaneously and men needing orchiopexy. Fertility potential is unknown for men after immediate surgery at diagnosis; this should be the subject for future studies.

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Chapter 6

Acquired undescended testes and fertility potential:

Is orchiopexy at diagnosis better than awaiting spontaneous descent?

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Summary

The aim of this study was to evaluate testicular function in men with previous acquired undescended testis (UDT) in whom orchiopexy was performed at diagnosis compared with a similar group of men in whom spontaneous descent was awaited until puberty.

Secondly, we examined the influence of age at orchiopexy on fertility parameters in adult life.

A total of 169 men of the 'orchiopexy at diagnosis'-group and 207 men of the 'wait-and-see'-protocol group were invited for participation.

All participants underwent an andrological evaluation, including medical history, physical examination, scrotal ultrasound, determination of reproductive hormones, and semen analysis.

Results were compared for men in whom orchiopexy was performed at diagnoses with men in whom spontaneous descent was awaited until puberty followed by orchiopexy in case of non-descent.

In the 'orchiopexy at diagnosis'- group 63 men, of whom 14 with bilateral UDT, and in the 'wait-and-see'-protocol group 65 men, of whom 15 with bilateral UDT, were included.

For unilateral UDT Inhibin B was found to be significantly lower and median progressive motility was higher in men with orchiopexy at diagnosis.

For bilateral UDT, semen concentration and progressive motility showed a trend towards a favourable outcome for orchiopexy at diagnosis.

Age at orchiopexy being under or above 10 years of age had no significant influence on the fertility potential.

The outcome of physical examination, scrotal ultrasound, endocrine function, and semen analysis indicates a compromised fertility potential in men with previous acquired UDT. None of the protocols proved to be superior. For bilateral UDT, a trend towards favourable outcome of orchiopexy at diagnosis was seen. Furthermore, age at orchiopexy did not have an influence

on fertility parameters. Therefore, in our opinion a 'conservative policy' is warranted for unilateral UDT, especially because over 50% of acquired UDT descend spontaneously.

Introduction

Undescended testis (UDT) is a common urogenital abnormality in boys and is associated with impaired spermatogenesis and an increased risk of testicular malignancy [1].

Based on previous testis position UDT can be categorized in congenital and acquired forms [2,3]. For congenital UDT, it is currently recommended to perform orchiopexy at 6-12 months of age [4]. However, it is often performed at a later age. Up to 2/3 of these 'late' orchiopexies concern boys with acquired UDT [5,6]. Surgical correction at diagnosis for acquired UDT is often advised [7]. However, in over 50% of boys with acquired UDT spontaneous descent occurs before puberty [8,9]. Therefore, in The Netherlands, a 'wait-and-see'-protocol is used in which spontaneous descent is awaited until puberty followed by orchiopexy in case of non-descent.

Little is known about fertility in men with acquired UDT and the effect of orchiopexy at diagnosis or the 'wait-and-see'-protocol. The aims of our study were two-folded. Firstly, we objectified the impact of orchiopexy at diagnosis in patients with acquired UDT on fertility parameters in comparison with men who had had a 'wait-and-see'-protocol for acquired UDT. In other words, is a 'wait-and-see'-protocol as used in The Netherlands safe, in terms of fertility potential, to diminish the number of orchiopexies for acquired UDT. Secondly, we examined the influence of age at orchiopexy, if operated upon, on fertility parameters in adult life.

Patients and methods

All patients were part of an historical cohort. Patients were treated with one of the two different treatment protocols depending on the hospital they visited. Patients were retrospectively included in this follow-up study. Data on fertility potential was prospectively collected.

Definition and exclusion criteria

Acquired UDT implied that a previous normal stable scrotal position was documented at least once either by the Regional Youth Health Care Institution, general practitioner, or hospital. The most caudal testicular position after manipulation had to be non-scrotal or unstable scrotal position at diagnosis.

Exclusion criteria were: previous ipsilateral inguinal or scrotal surgery, previous hormonal treatment, chromosomal abnormalities, and dysmorphic syndromes. Age at time of follow-up had to be > 18 years.

Participants

'Orchiopexy-at-diagnosis'-group

Between 1986-2009, boys with acquired UDT referred to the Medical Center Alkmaar and Juliana Children's Hospital, The Hague were operated at diagnosis by three experienced paediatric surgeons.

'Wait-and-see'-protocol group

Between 1982 and 2004, boys with acquired UDT referred to Erasmus Medical Center-Sophia Children's Hospital, Rotterdam, and the Medical Center, Alkmaar, were followed by three experienced physicians annually until puberty. Spontaneous descent was awaited until at least Tanner stage P2G2, after which orchiopexy was performed in case of non-descent. Findings at physical examination were registered on a standardized form. Orchiopexy was performed by paediatric surgeons with years of experience performing orchiopexies.

The group is described in detail in a recent article by van Brakel et al. [10].

Methods

In total 376 men (169 men from the 'orchiopexy-at-diagnosis'-group and 207 men from the 'wait-and-see'-protocol group) were recruited by post. The methods for recruitment and andrological evaluation were published previously [10,11]. Men declining participation were asked for their reasons to decline. Participation involved a single hospital visit at the Erasmus Medical Center Rotterdam, including medical history taking, physical examination, scrotal ultrasound, venous puncture, and semen sample delivery.

Medical history focused on risk factors for fertility problems other than cryptorchidism and to the proportion of men attempting fatherhood. Physical examination and scrotal ultrasound were performed to measure testicular volume and to observe the presence of testicular and epididymal abnormalities and varicocele. Testicular volume was measured with an automated

formula from the ultrasound machine; testicular volume = width x height x depth x 10^{-3} x 0.523* (*= 4/3π x ½ x ½ x ½). The reference value for normative testicular volume was set at 13.4 mL, based on previous studies in healthy adults [12-14]. Testicular volume of UDT was measured; in case of bilateral UDT the mean testicular volume was used. The following parameters were evaluated: Leydig cell function, represented by luteinizing hormone (LH) (reference value 1.5-8 IU/L) and testosterone (reference value ≥ 10 nmol/L), as well as Sertoli cell function, represented by follicle stimulating hormone (FSH) (reference value 2-7 IU/L) and Inhibin B (reference value ≥ 150 ng/L). The venous blood sample was taken, preferably before 10:00 AM and before 11:00 AM at the latest.

Semen samples were produced by masturbation after at least 3 days ejaculatory abstinence. The following reference values were considered as normal; semen volume ≥ 1.5 mL, sperm concentration ≥ 15x10*6/mL and progressive motility ≥ 32% [15].

Statistical analysis

Continuous variables were tested with the Mann-Whitney U-test; categorical variables with Fisher's exact test. First, characteristics of participants were compared with those of non-participants. Second, fertility parameters (testicular volume, endocrinological values, and semen parameters) were compared between the men previously treated in Medical Center Alkmaar and the men treated in the Juliana Children's hospital. We compared the two treatment protocols (orchiopexy at diagnosis vs. 'wait-and-see'-protocol) for uni- and bilateral UDT separately.

Multivariate logistic regression analyses served to test the relationship between these bivariate categories and their predictors; that is, the relationship between fertility parameters (bivariate classes: being below or above the reference value) and the predictor "type of treatment protocol". Known confounders such as uni-/bilateral UDT, smoking, varicocele and Body Mass Index (BMI) were corrected for in these analyses.

For all operated men from either treatment group (n=96), we compared men operated under the age of 10 years to men operated after the age of 10 years. Multivariate logistic regression analyses served to analyse the relationship of fertility parameters and the predictor "operation before or after the age of ten years". Type of treatment protocol was corrected for. For all analyses a two-sided *p*-value of less than 0.05 was considered statistically significant.

Ethics

The study was reviewed and approved by the Medical Ethical Review Committee at Erasmus University Medical Centre in Rotterdam (MEC No. 2004-206). Written informed consent was obtained from all participants.

Results

'Orchiopexy-at-diagnosis'-group

Sixty-three of 169 eligible men (37%) participated. Most non-participants mentioned time constraints and objection to the investigations as main reasons for refusal. Three men did not show up. For two men 'having fathered children' was a reason to decline participation. One man had no wish to father children and for that reason did not want to participate. Another man had known infertility (azoospermia) and refused to participate. There was no significant difference in age at recruitment, age at surgery or the proportion of bilateral UDT between participants and non-participants.

In total, we included 63 patients with 77 previously operated acquired UDT (14 bilateral, 49 unilateral). Median age at orchiopexy was 10.3 years (3.7 – 15.2). The reason for older age at orchiopexy was late diagnosis. One man refused to give blood.

'Wait-and-see'-protocol group

Sixty-five of 207 eligible men (31%) participated. Most men mentioned time constraints and objection to the investigations as main reasons for refusal. Six men did not show up. Two men reported that having fathered children was a reason to decline participation. One non-participant with bilateral acquired UDT with spontaneous descent replied that he had an azoospermia. There was no significant difference in age at recruitment, age at surgery or the proportion of bilateral UDT between participants and non-participants.

In total, we included 65 men with 80 acquired UDT. Spontaneous descent had occurred in 32 participants (24 unilateral and 8 bilateral). Median age at orchiopexy (N=33; 26 unilateral and 7 bilateral) was 13.2 years (range 4.7 - 17.8 years). Most important criteria for prepubertal orchiopexy were inguinal complaints or ipsilateral inguinal hernia. One man was unable to produce semen for the study and one man refused to give blood.

Unilateral UDT; orchiopexy at diagnosis vs. 'wait-and-see'-protocol (Table 1)

In the 'orchiopexy-at-diagnosis'-group age at orchiopexy was [median (range)] 10.1 years (3.7 – 15.2). The 'wait-and-see'-protocol group contained 50 men with unilateral UDT of whom 26 men had an orchiopexy as spontaneous descent did not occur. Age at orchiopexy was [median (range)] 12.9 years (4.8 – 17.8). Boys operated at diagnosis were significantly younger (*p*-value 0.02).

Seven men (14.3%) who underwent orchiopexy at diagnosis had a history of a venereal disease in comparison with none of the men after the 'wait-and-see'-protocol (*p*-value 0.01).

Physical examination and scrotal ultrasound

Men who had orchiopexy at diagnosis were significantly taller [median (range) 187.5 cm (169.0 – 203.0) vs. 184.5 cm (166.0 – 198.5); *p*-value 0.04], and had a lower BMI [median (range) 22.4 kg/m^2 (19.1 – 32.1) vs. 23.3 kg/m^2 (17.6 – 36.8); *p*-value 0.04], in comparison with men after the 'wait-and-see'-protocol.

Endocrine function

The only significant difference was found in Inhibin B levels, which was significantly lower in men with orchiopexy at diagnosis [median (range) 134.0 ng/L (44.0 – 278.0) vs. 161.0 ng/L (10.0 – 345.0); *p*-value 0.03].

Semen analysis

Progressive sperm motility was significantly higher in the group with orchiopexy at diagnosis [median (range) 47.5% (10 - 69) vs. 45.0% (3 - 75); *p*-value 0.03].

Bilateral UDT; orchiopexy at diagnosis vs. 'wait-and-see'-protocol (Table 2)

In the 'orchiopexy-at-diagnosis'-group age at orchiopexy was [median (range)] 10.7 years (4.9 – 12.8). The 'wait-and-see'-protocol group contained 15 men with bilateral UDT of whom 7 men had an orchiopexy as spontaneous decent did not occur. Age at orchiopexy was [median (range)] 13.2 years (7.7 – 15.1).

Men who had had bilateral UDT and orchiopexy at diagnosis did not attempt fatherhood significantly more often (6 out of 14 vs. 4 out of 15; *p*-value 0.45), however they did succeed significantly more often (6 out of 6 vs. 1 out of 4; *p*-value 0.03).

Physical examination and scrotal ultrasound

No significant differences were found in physical examination and ultrasound parameters. Testicular volume was smaller than the reference value of 13.4 mL in both treatment groups, median 8.5 mL (range 3.0 - 13.4) after orchiopexy at diagnosis; 10.9 mL (range 5.2 - 14.6) after the 'wait-and-see' protocol (*p*-value= 0.28).

Endocrine function and semen analysis

No significant differences were found when evaluating endocrine function and semen analysis. There was a notable, albeit insignificant difference in the median of concentration of spermatozoa of men operated at diagnosis, 22.0x10*6/mL (range 0-135), compared with median 8.2x10*6/mL (range 0.0-60.0) after the 'wait-and-see'-protocol (*p*-value= 0.18).

Multivariate regression analyses for treatment protocol

In Table 3, we compared all outcomes of men from the 'orchiopexy-at-diagnosis'-group with the 'wait-and-see'-protocol group for fertility parameters being the normal value, corrected for possible confounders that is, smoking, varicocele, BMI, uni- or bilateral UDT. A relative risk < 1.0 indicates a lower chance of having normal fertility parameters in the 'wait-and-see' group. The change of having a normal testicular volume (e.g. volume ≥ 13.4 mL) and normal Inhibin B level (e.g. ≥ 150 ng/L) are higher in the 'wait-and-see'-protocol group [(Testicular volume: relative risk (RR) 3.19; 95% confidence interval (CI) 1.24 – 8.2, *p*-value 0.02) and Inhibin B: RR 1.51; 95% CI 1.03 – 2.22, *p*-value 0.04]. The change of normal semen motility (≥ 32%) is higher in the 'orchiopexy-at-diagnosis'-group (RR 0.80; 95% CI 0.66 – 0.97, *p*-value 0.02).

The outcomes were comparable for the univariate regression analyses and the multivariate regression analyses. The changes of normal semen concentration (≥15 x 10*6/ml) or normal semen motility (≥ 32%) are higher in the group of men with a history of unilateral UDT. None of the other possible confounders were of significant influence on outcome.

Unilateral UDT; Influence of age at orchiopexy on fertility parameters using a cut-off of 10 years of age

Seventy-five men with unilateral UDT had an orchiopexy ('orchiopexy-at-diagnosis'-group N=49 and 'wait-and-see'-protocol group N=26). Thirty-two out of 75 had had orchiopexy before 10 years of age, at median age 6.7 years (range 3.7-9.9). Forty- three men were operated after 10 years at a median age of 12.8 years (range 10.0-17.8). Outcome at follow-up of physical examination, scrotal ultrasound, endocrine function, and semen analysis did not statistically differ between the age groups (data not shown).

Bilateral UDT; Influence of age at orchiopexy on fertility parameters using a cut-off of 10 years of age

Twenty-one men with bilateral UDT had had an orchiopexy ('orchiopexy-at-diagnosis'-group N=14 and 'wait-and-see'-protocol group N=7). Eight out of 21 men had had bilateral orchiopexy before 10 years of age. The median age at orchiopexy was 8.4 years (range 4.9-9.5). Thirteen men were operated on after 10 years at a median age of 12.4 years (range 10.4-15.1) (p-value < 0.005). Men operated on after the age of 10 had significantly larger testicular volume at follow-up than the men operated on before 10 years of age [median (range) 11.3 mL (3.4-13.4) and 8.0 mL (5.3-9.5) respectively (p-value < 0.05)] Outcome of physical examination, endocrine function and semen analysis did not statistically differ. There was a notable, albeit insignificant difference in the median semen concentration, i.e. median 8.0x10*6/mL (range 0.0-42.0) for men operated on bilaterally under the age of 10 vs. median 26.0x10*6/mL (range 0.0-135.0) for men operated after the age of 10 (p-value= 0.1)

Multivariate regression analyses for age at orchiopexy using a cut-off of 10 years of age (Table 4)

We combined both treatment protocols and compared men who had orchiopexy before the age of 10 years versus orchiopexy after the age of 10 years. Changes of having normative fertility parameters were not statistically different between the group who had surgery before the age of 10 years and the group who had surgery after the age of 10 years after correction for possible confounders, that is, treatment protocol, smoking, varicocele, BMI, uni- or bilateral UDT. For LH we could not perform the multivariate analyses since only 2 men had an LH above the reference value. The chance of normal testicular volume was lower with increasing BMI.

Additional analysis; Influence of age at orchiopexy without the outliers (Table 5)

Since the difference of age at orchiopexy between the two groups was smaller than previously expected, we performed an additional analysis in order to evaluate the effect of age at orchiopexy on fertility parameters. We compared the 'early' diagnosed boys of the 'orchiopexy-at-diagnosis'-group, e.g. operated before the age of 8 years, with 'older' patients of the 'wait-and-see'-protocol group operated after the age of 12 years. Hereby we excluded from the 'orchiopexy-at-diagnosis'-group, the boys that were referred late. We excluded from the 'wait-and-see'-protocol group, boys that had orchiopexy before puberty. Table 5 shows that no differences were found for neither of the fertility parameters for uni- or bilateral cases.

Discussion

To our knowledge, this is the first study evaluating fertility parameters in men with previous acquired UDT managed with either of two protocols, that is, orchiopexy performed at diagnosis or awaiting spontaneous descent until puberty. The outcome of physical examination, scrotal ultrasound, endocrine function, and semen analysis indicates a compromised fertility potential in men with previous unilateral or bilateral acquired UDT. However, differences between the two groups were small. For unilateral acquired UDT the 'wait-and-see'-protocol used in the Netherlands does not compromise fertility potential to a greater extent than does orchiopexy at diagnosis. For bilateral UDT, a trend towards favourable outcome of orchiopexy at diagnosis was seen, however, these results should be interpreted with caution as numbers were small. Furthermore, age at orchiopexy was not of influence on fertility parameters. None of the protocols proved to be superior, both with regard to unilateral and bilateral UDT. For unilateral UDT Inhibin B, which is an indicator for spermatogenesis was found to be significantly lower in men with acquired UDT and orchiopexy at diagnosis. However, sperm concentration was comparable between treatment groups. For

unilateral UDT, the median sperm concentration was above the WHO reference values of 15 x10*6/mL in both groups. Nevertheless, some men with sperm counts above the lower limit of the normal range, defined by WHO, may in fact be subfertile.

A population-based study of 430 first-pregnancy planners showed an increased probability of conception with increased sperm concentration up to 40 x10*6/mL [16]. For the range of sperm concentrations between 15 x10*6/mL and 40 x10*6/mL, small differences may be critical. Therefore, men with unilateral acquired UDT in both groups may in fact be subfertile. It is difficult to predict fertility based on semen analyses as paternity rates are also depending on fertility potential of the partner.

Alternately the difference in median progressive motility was higher in the 'orchiopexy-at-diagnosis'-group, nevertheless this was only 2.5%, and both median values were above 32%, which is the cut-off value for progressive motility according to the WHO manual [15]. Therefore, the significant differences that were found are not explicit and even alternately indicate better fertility potential in the group who had had immediate orchiopexy at diagnosis and men who had a 'wait-and-see'-protocol. Therefore, none of the protocols proved to be superior in terms of fertility potential.

For bilateral UDT, the only significant difference was found for successful attempt to fatherhood in favour of the 'orchiopexy-at-diagnosis'-group. Furthermore, semen concentration and progressive motility also showed a favourable outcome for the orchiopexy at diagnosis group. The wide range of sperm concentrations in this small group of men may have caused the striking but insignificant, difference in median concentration. The results between the bilateral groups should be interpreted carefully because of the small numbers.

In the multivariate regression analyses, we corrected for uni and bilateral UDT. The chance of a normal testicular volume and Inhibin B level was higher in the 'wait-and-see'- protocol group. Alternately, the chance of normal motility was higher in the 'orchiopexy-at-diagnosis'-group.

Orchiopexy and its impact on fertility has been studied extensively [17]. However, most studies did not differentiate between congenital and acquired UDT. There is increasing evidence that acquired UDT and congenital UDT share the same aetiology [18,19]. Our results seem to confirm this, implicating that acquired UDT is not a variant of normally descended testes without pathological consequences. In the present study, fertility potential appeared to be compromised in men with previously acquired UDT. Median testicular volume in all evaluated groups was smaller than the norm value of 13.4 mL [12-14]. For reproductive hormones, median Inhibin B was lower than 150 ng/L for both unilateral and bilateral UDT after orchiopexy at diagnosis and for men with bilateral UDT who had the 'wait-and-see'-protocol. Semen analysis showed that oligozoospermia occurred in 27 and 43% of, respectively the uni- and bilateral

group who had orchiopexy at diagnosis. After the 'wait-and-see'-protocol, oligozoospermia occurred in 38 and 67% of, respectively, the uni- and bilateral group. Astenospermia was found in 11% of the uni- and in 23% of the bilateral group who had orchiopexy at diagnosis and in, respectively, 22 and 57% of the uni- and bilateral group after 'wait-and-see'-protocol. This study shows that fertility parameters were impaired with respect to cut-off values used in the literature, irrespective of the protocol used.

Fewer fertility problems were expected in acquired UDT because the crucial development of type A spermatogonia for future fertility has probably already occurred during the first year of life before the ascent occurs [1,17]. However, biopsies of acquired UDT showed similar histopathological changes as biopsies of congenital UDT [19]. So far, no evidence is available that fertility improves with treatment at diagnosis in acquired UDT. The recommendation of surgical correction at the time of diagnosis for acquired UDT is mainly based on the supposed negative influence of the thermal inguinal environment on testicular development and future spermatogenesis [3,20,21]. Conversely, a conservative attitude for acquired UDT has also been advocated, restricting orchiopexy to cases of non-descent at puberty. At puberty, spontaneous descent will occur in 50-75% of the patients [8,9,22]. In such cases, surgery is redundant. In the study group of the 'wait-and-see'-protocol spontaneous descent had occurred in 50% of the included men. Fertility potential did not differ between men who had spontaneous descent and men who underwent orchiopexy after the 'wait-and-see'-protocol [10].

Regarding unilateral UDT, age at orchiopexy in the 'orchiopexy-at-diagnosis'-group was significantly lower than that in the 'wait-and-see'-group, although the difference in median age at orchiopexy was relatively small. Some men in the former group were relatively old at diagnosis, whereas in the latter group ,some were at prepubertal age at orchiopexy. Reasons to perform prepubertal orchiopexy were inguinal complaints or ipsilateral inguinal hernia [10]. Apparently this is the clinical practice in which acquired UDT can become obvious at later age and on the other hand reasons for performing orchiopexy at prepubertal age despite the intention to await spontaneous descent. As the difference of age at orchiopexy between the two groups (egg., 'orchiopexy-at-diagnosis'-group and 'wait-and-see'-group) was smaller than previously expected, we performed an additional analysis in order to evaluate the effect of age at orchiopexy on fertility parameters. We compared the 'early' diagnosed boys of the 'orchiopexy-at-diagnosis'-group, for example, operated before the age of 8 years, with patients of the 'wait-and-see'-protocol group operated after the age of 12 years. No differences were found for neither of the fertility parameters. When evaluating fertility parameters between men operated before and after the age of ten from the complete group of men who had had orchiopexy, we only found that men with bilateral UDT operated after the

age of 10 years had a higher testicular volume. In the multivariate analysis normative values were not significantly more often found in either group.

In the literature variable effects of younger age at orchiopexy in congenital UDT are documented. While some studies suggest a positive effect on fertility parameters later in life [23-25], others cannot find a favourable effect of early treatment [11,26-29]. To our knowledge, no previous studies have examined the effect of age at orchiopexy in boys with acquired UDT.

This study addresses fertility potential in men with acquired UDT. The risk of testicular cancer and especially the potential different risk of developing a testicular malignancy between the two treatment protocols fall out of the scoop of this article.

Several limitations of this study need to be addressed. Unfortunately this study is not a randomised controlled trial. We included patients from two historical cohorts, treated with two different protocols. Prospectively patients were included for follow-up, comparing long-term outcome of both treatment protocols.

Participation rates of the two protocol groups (33 vs. 30%) were comparable, also with earlier published studies [11,30]. Reasons for non-participation may have introduced a selection bias. However, this selection bias can give either an overestimation or an underestimation of fertility potential as suspected infertility or prolonged intent to attempt fatherhood can be a reason for both participation and refusal. Therefore, we presume that the risk for selection bias because of fertility problems is the same in both protocol groups.

The difference of age at orchiopexy between both treatment protocols was small,

despite the aim to perform immediate orchiopexy at diagnosis in one treatment protocol. This observation underlines that in clinical practice patients with acquired UDT may be diagnosed at later age for the first time. Another confounding factor is the unknown time between acquirement of UDT and diagnosis, those who were operated soon after diagnosis may have had UDT for a longer time.

Using paternity for the evaluation of fertility potential is another limitation as it has shortcomings; it measures the function of the whole male and female fertility potential. Besides, a presumed father may not be the biological father.

Lastly, each man produced only one sperm sample for analysis and we are therefore not informed about the biological variation of semen quality. However, a recent study has shown substantial agreement between consecutive semen analysis in young men [31].

Conclusion

Fertility potential in men with a history of acquired UDT in whom spontaneous descent is awaited until puberty is compromised to a degree comparable as in men in whom orchiopexy at diagnosis is performed. Our results for unilateral UDT illustrate that acquired UDT merely represents part of a spectrum of UDT and that orchiopexy at diagnosis does not seem to restore the long-term outcome on fertility parameters. Age at orchiopexy, which was relatively late in both groups, did not seem to have influenced fertility potential later in life. As a result of several confounding factors in our study, no strong recommendations can be made based on these data for neither treatment, in our opinion a 'conservative policy' is warranted for unilateral UDT, because over 50% of acquired UDT descend spontaneously and orchiopexies can be omitted. For bilateral UDT, although no significant differences were found in favour of either of the groups, we cannot advice for a 'conservative policy' as these results are based on small numbers.

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Part III
Evaluation of endocrine function and semen quality using experimental methods

Chapter 7

INSL3 and AMH in patients with previously congenital or acquired undescended testes

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Abstract

In previous reports no difference in Leydig and Sertoli cell function was found between congenital undescended testis (CUDT) and acquired UDT (AUDT) on basis of levels of luteinizing hormone, testosterone, follicle stimulating hormone or inhibin B. The present study was set out to detect differences in Leydig and Sertoli cell function between CUDT and AUDT using Insulin-like peptide 3(INSL3) and Anti-Müllerian hormone (AMH) serum levels.

Serum samples of 118 men with a history of UDT (CUDT N=55 of whom 6 bilateral, AUDT N=63 of whom 15 bilateral) were investigated. Firstly, differences between CUDT and AUDT were analysed. Secondly, influence of age at surgery in CUDT and effect of spontaneous descent or orchiopexy in AUDT were evaluated.

For INSL3, no significant differences were found between the various groups of patients. However the bilateral congenital UDT group was very small. AMH was significantly higher in men with unilateral CUDT who had their orchiopexy before 24 months of age compared with after 24 months of age (16.8 (3.9 – 43.7) (median and range) vs 10.1 (2.4 – 40.4) µg/l, p=0.02). AMH levels in bilateral CUDT were significantly lower compared with bilateral AUDT (6.4 (1.7 – 11.4) vs 13.2 (6.1 – 30.1) µg/l, p=0.02). When comparing unilateral CUDT with bilateral CUDT, AMH levels in unilateral CUDT were significantly higher (12.1 (2.4 – 43.7) vs. 6.4 (1.7 – 11.4) µg/l, p=0.02).

It is concluded that no differences in Leydig cell function between the different UDT groups were found on basis of INSL3 levels. Sertoli cell function evaluated by AMH, was more negatively affected in bilateral CUDT in comparison with bilateral AUDT and in bilateral CUDT in comparison with unilateral CUDT. Moreover, in unilateral CUDT earlier orchiopexy positively influenced Sertoli cell function.

Introduction

Undescended testis (UDT) is a frequent urogenital abnormality in boys and can be of congenital or acquired origin [1-3]. In congenital UDT a stable scrotal position was never reached. Acquired UDT is defined as a palpable UDT that previously had a normal scrotal position, as documented by a physician. Acquired UDT is seen in 1-3% of boys during childhood [4,5] and its aetiology, natural course, and treatment are still debated. The proportion of (pre) pubertal spontaneous descent in boys with acquired UDT is between 57% and 71% [6,7]. Men with a history of UDT have a higher risk of developing impaired fertility later in life [8,9]. It was suggested that men with acquired UDT might have fewer fertility problems, since their testes initially descended and the crucial development of type A spermatogonia had probably already occurred during the first year of life before the testes ascended [10]. However, in our previous long-term follow-up study no statically significant differences in traditional fertility parameters were seen between men with congenital and acquired UDT [11]. In that study, we evaluated Leydig cell function by measuring serum levels of luteinizing hormone (LH) and testosterone. Serum follicle stimulating hormone (FSH) and inhibin B (InhB) were used to evaluate Sertoli cell function. In the present study we more extensively evaluated endocrinological function (e.g. Insulin-like peptide 3 (INSL3), Anti -Müllerian hormone (AMH), sex hormone binding globulin (SHBG), oestradiol) in the same group of patients to discover whether more subtle differences in Leydig cell and Sertoli cell function between different patient groups with UDT can be found. These endocrinological results were not reported previously.

INSL3, produced by Leydig cells, is essential for the abdominal part of testicular descent [12-14]. At adult age, INSL3 has been suggested to be a more sensitive parameter to detect impaired Leydig cell function than testosterone, since men with hypospermatogenesis had normal levels of testosterone and LH but reduced INSL3 levels [15-19]. AMH is produced by Sertoli cells. Lower serum AMH levels were found in subfertile men [20]. In healthy men, serum AMH cannot discriminate between fertile and infertile men; serum AMH does not show a correlation with impaired spermatogenesis [21,22]. However in men with UDT, AMH has been suggested to be a marker for Sertoli cell function [23]. In the present study we set out to detect differences in Leydig and Sertoli cell function between congenital and acquired UDT on basis of INSL3 and AMH serum levels. In addition, we evaluated the influence of age at surgery in men with congenital UDT on these parameters as well as their association with spontaneous descent or orchiopexy in acquired UDT. Also correlations of INSL3 and AMH with levels of other hormones of the pituitary-testis axis, semen parameters and testicular volume were investigated. To our knowledge, this is the first study to evaluate these specific hormone levels in adult men with a history of congenital or acquired UDT.

Material and methods

Patients

Men with a history of congenital or acquired UDT who participated in two long term follow-up studies on fertility potential performed at the Erasmus Medical Center were included retrospectively [11,24]. Data on hormone concentrations were prospectively collected between 2005 and 2010. Inclusion and exclusion criteria of participants in these studies were described previously [11,24]. Figure 1 shows an overview of included men. In the present study only men with two testes at follow-up were included. In short: The congenital UDT group (N=56, of whom 6 bilateral UDT) consisted of men who participated in a study evaluating fertility potential after treatment [24]. Subjects in that study were part of a historical cohort of boys who underwent orchiopexy during childhood at various ages and boys operated on before two years of age [25]. Median age at orchiopexy was 3.3 years (range 0.1 to 14.6 years). The acquired UDT group (N=64, of whom 15 bilateral UDT) consisted of men who participated in a study evaluating fertility potential in men with previously acquired UDT after follow-up during childhood [11]. Subjects in that study were part of a historical cohort with men with acquired UDT who had annual follow-up examination until puberty [6]. For acquired UDT this implies that a previously normal stable scrotal position was documented at least once at birth and/or at follow-up either by the Regional Youth Health Care Institution, general practitioner, or medical specialists. Furthermore, at inclusion the testis had to be palpable without ipsilateral inguinal clinically present pathological findings or previous ipsilateral inguinal surgery, and the most caudal testicular position after manipulation had to be non-scrotal or unstable scrotal. Spontaneous descent was awaited until at least Tanner stage P2G2 and followed by orchiopexy in case of non-descent. Spontaneous descent had occurred in 32 participants, of whom 8 had had bilateral UDT. Median age at orchiopexy in 32 participants (25 unilateral and 7 bilateral) was 13.3 years (range 4.75 to 17.8 years). For both historical cohorts maximum effort was taken to ascertain previous testicular position at inclusion for the initial study [6,25].

Blood analysis

A venous blood sample was taken, preferably before 10:00 AM and before 11:00 AM at the latest. Serum samples were assayed for INSL3 using radioimmunoassay (Phoenix Pharmaceuticals, Burlingame, CA, USA). AMH was measured using an in-house enzyme immunometric assay (Gen II, Beckman-Coulter, Webster, TX, USA) as previously described [26], LH, FSH and sex hormone binding globulin using fluorescence-based immunometric methods (Immulite 2000, Siemens-DPC, Los Angeles, CA, USA), testosterone and oestradiol using coated tube based radioimmunoassays (Siemens-DPC), and InhB using an enzyme-immunometric method (Oxford BioInnovation, Oxford, UK). Reference ranges for all hormone

concentrations are given in Table 1. All reference ranges were from the laboratory where the assays were performed, with the exception of those for INSL3, where literature data obtained with the same assay were used [15] . Intra- and interassay coefficients of variation for the various assays were as follows: INSL3 <9%, AMH < 5 and <10%, LH < 5 and <11%, FSH <3 and <8%, SHBG < 4 and <5%, testosterone <3 and <5%, oestradiol < 5 and <7%, and InhB <9 and <15% , respectively. Concentrations of non-SHBG bond testosterone and oestradiol were calculated using the method described by de Ronde et al. with a fixed concentration for albumin of 42 g/l [27].

Semen analysis

Semen samples were analysed according to the WHO-manual of 2010 [28]. The following reference values were considered as normal; semen volume ≥ 1.5 ml, sperm concentration ≥ $15*10^6$/ml, total sperm count ≥ $39*10^6$/ejaculate, progressive motility ≥ 32%, and ≥ 4% normal sperm morphology.

Statistics

Differences in hormone levels between different groups were analysed using the Mann-Whitney U test. Firstly, we compared hormone levels between the congenital UDT group and acquired UDT group. Secondly, we evaluated the influence of age at surgery in the congenital group by comparing men who were operated before 12 months of age vs after 12 months of age, before 18 months of age vs after 18 months of age, and before 24 months of age vs after 24 months of age. Lastly, in the acquired group we compared men who had spontaneously descended UDT with men who needed orchiopexy. We performed these analyses for unilateral and bilateral UDT separately. Subsequently, we compared hormone levels in the groups with unilateral and bilateral UDT with each other in the congenital and the acquired UDT group. Furthermore, we evaluated correlations between the levels of INSL 3 and AMH and the concentrations of the other hormones, semen parameters, and total testicular volume using Spearman Rank Correlation test followed by partial correlation analyses. For all analyses a two-sided p-value of less than 0.05 was considered statistically significant. Statistical analysis was performed with IBM SPSS Statistics version 21.

Results

Figure 1 shows an overview of included men and missing data. When comparing hormone concentrations in our patients with the reference ranges the following results were notable: in 4 out of the 96 men (4.2%) in whom INSL3 was known, the INSL3 level was normal, all others had INSL3 levels below the lower limit of the reference range. Over 80% of men had a normal AMH level (unilateral congenital UDT N=39 (80%), bilateral congenital UDT N=5 (83%), unilateral acquired UDT N=39 (80%), and bilateral acquired UDT N=13 (87%). FSH was above the reference range in approximately half of the patients with bilateral UDT (congenital UDT N=3 (50%), acquired UDT N=7 (47%)) in comparison with approximately 30% in the unilateral groups (congenital UDT N=14 (29%), acquired UDT N=15 (31%). InhB levels were more often below the lower limit of the reference range in the bilateral UDT groups than in the unilateral UDT groups and more often in the congenital groups than in the acquired groups (unilateral congenital UDT N=23 (47%), bilateral congenital UDT N=4 (67%), unilateral acquired UDT N=19 (39%), and bilateral acquired UDT N=8 (53%)).

Unilateral UDT

Congenital UDT vs Acquired UDT (Table1)

Oestradiol levels in congenital UDT patients were significantly higher compared with those in the acquired group ($p = 0.005$). All other hormone concentrations, including non-SHBG bound oestradiol, did not show statistically significant differences.

Congenital UDT; influence of age at orchiopexy (Table 2)

AMH was significantly higher in men who had had their orchiopexy before the age of 24 months ($p = 0.02$). LH was significantly higher in men operated before the age of 18 months ($p = 0.03$). Oestradiol was significantly lower in men operated at younger age (orchiopexy <12 months vs orchiopexy >12 months: $p = 0.001$; orchiopexy < 18 months vs orchiopexy > 18 months: p-value <0.001; and orchiopexy < 24 months vs orchiopexy > 24 months: $p = 0.009$). Non-SHBG bound oestradiol, did not show statistically different values.

Acquired UDT; spontaneous descent vs orchiopexy (Table 3)

LH was significantly higher in men who had had a spontaneous descent in comparison with men who needed orchiopexy ($p = 0.04$). None of the other measured hormones showed statistically different concentrations.

Bilateral UDT

Congenital UDT vs Acquired UDT (Table 1)

AMH levels in congenital UDT were significantly lower compared with those in the acquired group (p = 0.02). Non-SHBG bound testosterone levels in men with congenital UDT were significantly lower compared with those in the acquired UDT group (p = 0.01). None of the other measured hormones showed statistically different concentrations

Congenital UDT; influence of age at orchiopexy

None of the measured hormones showed statistically different concentrations when analysing the different age groups (data not shown). However, we included only 6 men with bilateral congenital UDT, of whom one patient had been operated at 17 months of age and the other 5 patients after 24 months of age.

Acquired UDT; spontaneous descent vs orchiopexy (Table 3)

No significant differences were found in hormone concentrations between men whose testis descended spontaneously in comparison with men who needed orchiopexy.

Unilateral versus bilateral UDT (Table 1)

Only in the congenital group significant differences were found comparing the unilateral UDT group with the bilateral UDT group. AMH levels in the unilateral UDT group were significantly higher compared with the bilateral group (p = 0.02). Non-SHBG bound testosterone levels in the unilateral group were significantly higher compared with those in the bilateral group (p = 0.02). In the acquired group no significant differences between unilateral and bilateral UDT were found.

Correlation between concentration of INSL3 and AMH with the other hormone levels, semen parameters, and total testicular volume

Levels of INSL3 were positively correlated with LH (N=96, correlation coefficient (CC) 0.22, p = 0.03), FSH (N=96, CC 0.233, p = 0.02), testosterone (N=96, CC 0.303; p = 0.003), non-SHBG bound testosterone (N=95, CC 0.332, p = 0.001), and negatively with InhB (N=96, CC -0.234, p = 0.02). No significant correlation was found when performing partial correlation between

INSL3 and LH after correction for FSH or between INSL3 and FSH correcting for LH. A significant correlation between INSL3 and testosterone was found when performing partial correlation correcting for LH (Correlation 0.22, degrees of freedom 93; p = 0.03).

AMH was negatively correlated with LH (N=116, CC -0.185, p = 0.05), FSH (N=116, CC -0.47; p = <0.001), oestradiol (N=115, CC -0.208; p = 0.03),and positively with InhB (N=116, CC 0.413; p <0.001), semen concentration (N=114, CC 0.239; p = 0.01), semen motility N=109, CC 0.249; p = 0.009), and total testicular volume (N=116, CC 0.394; p <0.001). A significant correlation was found when performing a partial correlation for AMH and FSH correcting for LH (Correlation –0.362, degrees of freedom 113; p <0.001). No significant partial correlation was found for AMH and LH when correcting for FSH. After performing a partial correlation for AMH and InhB corrected for FSH no significant correlation was found. This was also seen for the correlation between AMH and semen concentration corrected for InhB.

Discussion

In this study we evaluated Leydig and Sertoli cell function using INSL3 and AMH respectively. The concentrations of these hormones might reflect functional status of both Leydig and Sertoli cells more accurately than LH, testosterone, FSH, and InhB, which we used in our previous study and therefore might uncover more subtle differences between congenital and acquired UDT. In our previous study no explicit differences were found in semen parameters when comparing different congenital and acquired UDT groups [11,24]. However, we did find that bilateral UDT patients were more negatively affected with regards to semen parameters than men with previously unilateral UDT. In the present study, INSL3 showed impaired Leydig cell function for both congenital and acquired UDT. However, INSL3 could not discriminate between the different UDT groups. In bilateral congenital UDT in comparison with bilateral acquired UDT Sertoli cell function analysed on basis of AMH was more negatively affected. This was also the case for bilateral congenital UDT in comparison with unilateral congenital UDT. AMH showed a possible advantage for early surgery in unilateral congenital UDT.

Testicular descent is mostly described as a biphasic process with a transabdominal and an inguinoscrotal phase [14,29]. INSL3 controls the transabdominal phase [12-14]. Age-related patterns of testosterone and INSL3 are comparable: a small increase during mini puberty, very low during childhood, increasing during puberty and high levels in adults [30]. Foresta et al. found that INSL3 was lower in men with testicular damage involving Leydig cells and men with hypospermatogenesis when compared with normal adult men [15]. In the present study, although most INSL3 levels were low no significant differences were found in INSL3 levels between the different UDT groups. However, this data should be interpreted with caution

since the groups are small, especially the bilateral groups, and this could possibly cause false negative conclusions. Non-SHBG bound testosterone in men with bilateral congenital UDT had significantly lower concentrations compared with men with bilateral acquired UDT, possibly indicating that Leydig cell function in congenital UDT is more affected than in acquired UDT. In the congenital group men with unilateral UDT had higher non-SHBG bound testosterone compared with men with bilateral UDT. This may indicate that Leydig cell function in bilateral UDT is more affected than in unilateral UDT in congenital UDT. We found significant positive correlations between INSL3 and LH, testosterone, and non-SHBG bound testosterone and a negative correlation with InhB. Foresta et al. described a positive correlation between INSL3 and LH and testosterone in normal men [15]. Bay et al. found a positive correlation between INSL3 and testosterone in the general population and in men with normospermia but not in infertile men when evaluating correlations between INSL3 with LH, FSH, testosterone and InhB [31]. However, they found no correlation between INSL3 and semen parameters conform our data [31]. Since INSL3 correlates with testosterone and LH and not with semen parameters in normal men and men with a history of UDT, the additional clinical value of INSL3 to evaluate Leydig cell function is questionable.

AMH is secreted by immature Sertoli cells and is responsible for regression of de Müllerian ducts [32]. In boys, AMH steeply increases during the first few months of life, followed by a decline to a stable relatively high level until puberty. Under the influence of testosterone, between Tanner stage T2 and T3 AMH levels rapidly decline to a low stable level in adulthood [33-35]. In adult men, AMH levels are indicative for the total number of Sertoli cells [34,36]. Lower AMH serum levels are found in subfertile men in comparison with controls [20]. In the congenital UDT group we found that men with unilateral UDT had higher AMH levels than those with bilateral UDT and in the unilateral congenital UDT group AMH was significantly higher in men operated before the age of 24 months. This implies that Sertoli cell function at adult age is better when patients with congenital UDT are operated at younger age. This, however, did not result in statistically significant differences in FSH, InhB and semen parameters as was shown previously [24]. When comparing the UDT groups, the bilateral congenital UDT group had significantly lower AMH levels compared with the bilateral acquired UDT group. Sertoli cell function in congenital UDT is apparently more severely affected than in acquired UDT. This is also the case for bilateral UDT vs. unilateral UDT. However, the data on AMH levels in bilateral UDT should be regarded with caution since only 6 men were included with bilateral congenital UDT. In the literature, as in our study, a negative correlation between AMH and FSH and a positive correlation between AMH and InhB, semen concentration, motility and total testicular volume in serum from fertile men, infertile men and men with UDT is found [23,37]. Plotton et al. described that AMH was negatively correlated with FSH and positively with InhB, total testicular volume, and bioavailable testosterone in patients with genetic spermatogenic failure but not in patients with cytotoxic azoospermia [38]. These observations lead to the suggestion,

that AMH is a measure of the number of Sertoli cells present, whereas InhB depends on both Sertoli cell number and the quality of spermatogenesis. In this way, low numbers of Sertoli cells will lead to decreased AMH levels, as found in the bilateral congenital UTD patients and in the unilateral congenital UTD patients operated after 24 months of age. In a review by Sharpe et al. two periods of increasing Sertoli cell numbers before adulthood are described: the first is until approximately 18 months of age and the second just before puberty [39]. In our results boys operated after 24 months had lower AMH levels at adult age compared with boys operated before the age of 24 months, possibly indicating a defect in proliferation of Sertoli cells when the testis is non scrotal during the first months of life.

The present study has some shortcomings. The groups, especially bilateral UDTs are relatively small. For example, for bilateral congenital UDT only 6 men were included. We are aware that hard statistically significant conclusions cannot be made. We advise further evaluation of AMH in a bigger group of men with a history of UDT to confirm our results. Furthermore, due to logistical problems INSL3 measurements were not performed in all patients. Also, we have no reference values for INSL3 from our own laboratory but used data from the literature. Despite this we think this study adds to the current literature for the following reasons: [1] it is the first study to report on both INSL3 as well as AMH in a group of adults with a history of congenital or acquired UDT, [2] to our knowledge this is the largest study regarding INSL3 and AMH in men with UDT, [3] it reports on a group of patients with congenital and acquired UDT instead of a group with UDT in whom previous testis position has not been taken into account, and [4] uni- and bilateral UDT are considered separately.

In conclusion, Leydig cell function evaluated by INSL3 showed impairment for congenital and acquired UDT but did not discriminate between the different UDT groups. Sertoli cell function evaluated using AMH, was more negatively affected in bilateral congenital UDT in comparison with bilateral acquired UDT and in bilateral congenital UDT in comparison with unilateral congenital UDT. Moreover, in unilateral congenital UDT earlier orchiopexy positively influenced Sertoli cell function.

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Chapter 8

Sperm DNA damage measured by SCSA in men with a history of undescended testes.

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Abstract

Introduction

Previous follow-up studies showed no statistical differences in traditional fertility parameters between men with congenital undescended testis (CUDT) and men with acquired undescended testis (AUDT). In this study we evaluated differences in sperm DNA damage between CUDT and AUDT using Sperm Chromatin Structure Assay (SCSA). In addition, we evaluated the influence of age at orchiopexy in men with CUDT and the difference between men whose testis descended spontaneously and men who needed orchiopexy after a 'wait-and-see'-protocol (e.g. awaiting spontaneous descent until puberty and perform an orchiopexy in case of non-decent) in AUDT on sperm DNA damage.

Material and methods

In 50 men with CUDT and 49 men with AUDT the DNA fragmentation index (DFI) was measured. 22 healthy proven fertile men served as controls. Decreased fertility potential was considered if DFI was above 30%.

Results

Sperm DNA damage in CUDT and AUDT was not statistically different. DFI was higher in the complete group of congenital UDT and men with bilateral congenital UDT in comparison with a control group. Age at orchiopexy in CUDT had no statistical effect on DNA damage. In AUDT no difference was found in DFI between men needing an orchiopexy and men with a spontaneous descent.

Conclusions

This study supports the hypothesis that UDT is a spectrum containing congenital UDT and acquired UDT. Early treatment during childhood for congenital UDT and the outcome of a 'wait-and-see'-protocol for acquired UDT has no influence on sperm DNA damage at adult age.

Introduction

Undescended testis (UDT) is a common urogenital abnormality. A distinction into two different types of UDT can be made [1,2]. Approximately, 1% of boys at the age of one year have a congenital UDT [3]. Moreover, 1-3% of boys with a previously normally descended testis develop UDT during childhood, an acquired UDT [4,5]. Men with a history of UDT have a higher risk of developing impaired fertility later in life [6,7]. Little is known about the differences in fertility potential between patients with congenital UDT and acquired UDT. In our previous long-term follow-up study no statically differences in traditional fertility parameters were seen between men with congenital and acquired UDT [8]. Therefore, we hypothesized that acquired UDT and congenital UDT share the same aetiology.

Conventional semen analysis is the routine test to diagnose sub- or infertility in males. However semen analysis has limitations such as inter-individual variations as well as large inter- and intralaboratory variability [9,10]. Moreover classic semen parameters are poor predictors for fecundity [11]. Semen quality can also be evaluated by determining sperm DNA damage. A review showed that sperm DNA damage testing can be used as a discriminator for fertility potential, since the level of sperm DNA damage correlates with spontaneous pregnancy and results of intra-uterine insemination [12]. Also, the biological variation of sperm DNA fragmentation is lower than conventional semen parameters [13].

In this study we aimed to explore the differences in sperm DNA damage between congenital and acquired UDT, using Sperm Chromatin Structure Assay (SCSA). Since in our previous studies no differences in traditional fertility parameters were found, we hypothesize that we will not find differences in the level of sperm DNA damage between the different groups either. In addition, we evaluated the influence of early or late orchiopexy in men with congenital UDT on sperm DNA damage levels, as well as the difference between men whose testis descended spontaneously and men who needed orchiopexy in acquired UDT. Finally, the sperm DNA levels in men with a history of UDT attempting fatherhood were evaluated. To our knowledge, this is the first study to determine fertility potential, by using SCSA, in men with a history of congenital and acquired UDT.

Material and Methods

Participants

Men with a history of congenital or acquired UDT who participated in two long term follow-up studies on fertility potential performed at the Erasmus Medical Center were included [8,14].

These men were invited to visit the andrology outpatient clinic for fertility screening, which included medical history taking, physical examination, scrotal ultrasound, determination of reproductive hormones, and semen analysis. Inclusion of participants in both studies was described previously. In short: the congenital UDT group (N=62) consisted of men who were treated for congenital UDT during childhood by orchiopexy at various ages [14]. Participants in the acquired group (N=65) were men who were diagnosed with acquired UDT as a child for which they were referred to the Erasmus Medical Center-Sophia Children's Hospital and Medical Center Alkmaar [8]. They were offered follow-up annually until puberty. Spontaneous descent was awaited until at least Tanner stage P2G2 and followed by orchiopexy in case of non-descent. Acquired UDT was defined as an UDT for which at least twice youth health care physicians had documented a previous scrotal position.

Twenty-two healthy proven fertile men served as controls and donated a semen sample for sperm DNA fragmentation prior to vasectomy. The study was reviewed and approved by the Medical Ethical Review Board at Erasmus University Medical Center in Rotterdam (MEC number 2004-206). Written informed consent was obtained from all men who agreed to participate in the study.

Semen analysis

A semen sample was produced by masturbation after a 3-5 day ejaculatory abstinence. These samples were analysed according to the WHO-manual of 2010 [15]. Within one hour, after liquefaction, sperm concentration (reference value ≥15 10*6/ml), total sperm count (reference value ≥39 10*6/ ejaculate), progressive motility (reference value ≥32%), and morphology (reference value ≥4% normal forms) were determined.

Sperm Chromatin Structure Assay (SCSA)

For the SCSA analysis part of the ejaculate was stored at -80°C. Sperm DNA fragmentation was assessed using SCSA previously described by Evenson and Jost [16]. A FACScan from Becton Dickinson (San Jose, CA, USA) was used to perform the SCSA. Frozen semen samples were quickly liquefied in a warm water bath of 37°C and diluted to a concentration sperm cells of 1 - 2x10^6/ml. After exposure to acid detergent solution the sample was stained with acridine orange. Three minutes after adding the acid detergent solution, data collection of the fluorescence patterns in 5000 cells was done. As recommended by Evenson and Jost bacteria, leucocytes and debris were removed from the total cell count during acquisition. A reference

sample was used to adjust the voltage gains of the flow cytometer FL3 and FL1 photomultipliers that analyse red and green fluorescence respectively before the actual sample and after every 5 - 10 samples. The reference sample was used to adjust the voltage gains to acquire stable mean red (X) and green (Y) values at 110 and 370 channels, respectively, with a maximum discrepancy of five channels. If the fluorescent signal of the reference sample drifted the voltage gains were re-adjusted. The DNA fragmentation index (DFI) expresses the amount of sperm DNA damage and is a reflection of red fluorescence to total fluorescence. The DFI was calculated using Cell Quest Pro and Winlist software. Each sample was measured twice and the mean of both DFI's was used for analysis. Decreased fertility potential was considered if DFI was above 30% [17,18].

Statistics

Mann-Whitney U test and Fisher's exact test

Continuous variables were tested with the Mann-Whitney U test; categorical variables with Fisher's exact test. When analysing the level of sperm DNA damage the median DFI was used as a continuous variable and furthermore the cut off value of 30% was used to dichotomize the DFI. The difference in DFI was analysed between participants with congenital and acquired UDT and a control group. Also the influence of age at orchiopexy before and after 12 months, 18 months and 24 months of age in the congenital group, and the effect in the acquired group of orchiopexy or spontaneous descent was evaluated. Furthermore the difference in DFI between men who succeeded in attempting fatherhood was compared with men who were unsuccessful.

Logistic regression analysis

To evaluate the effect of the type of UDT (congenital or acquired) on sperm DNA damage we modelled the percentage of sperm DNA damage as a function of other measured or otherwise known variables. We performed a logistic regression model in which sperm DNA damage is categorized as poor fertilizing potential or normal fertilizing potential using the 30% threshold for the percentage of DNA damage.

We corrected for age, smoking, varicocele, FSH, inhibin B, leucocytes in the ejaculate, days of ejaculatory abstinence, BMI, sperm concentration, sperm motility, and sperm morphology [19-28]. A backwards elimination procedure was used to delete insignificant variables (using a *p*-value threshold of 0.2). Another independent variable was uni-/bilateral UDT. For all analyses a two-sided *p*-value of less than 0.05 was considered statistically significant. The statistical

analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, Il) and with Stata Data Analysis and Statistical Software v.11.0 (StataCorp, College Station, TX, USA).

Results

Figure 1 shows the inclusion of 127 men for this study including the reasons why in 28 men the DFI was unknown (Figure 1). In 99 men the DFI was successfully measured in duplo (congenital group N=50 and acquired group N= 49). Table 1 shows the traditional semen analyses for the congenital group (N=62) and the acquired group (N=63, 2 men excluded due to no ejaculate), unilateral and bilateral UDT separately. The median values of the traditional semen parameters were around WHO reference cut-off values in both unilateral groups. Median concentration and total sperm count in the bilateral groups were clearly below the reference values in both groups. This was less clear for motility and morphology. No statistically significant differences in sperm parameters were found when comparing unilateral congenital UDT with unilateral acquired UDT or bilateral congenital UDT with bilateral acquired UDT. However, median concentration and total sperm count was remarkably lower in the bilateral congenital group in comparison with the bilateral acquired group.

Congenital UDT vs. acquired UDT (Table 2)

We found no statistically significant differences in median DFI levels or the percentage of men having a DFI >30% between congenital and acquired UDT when evaluating all UDT, unilateral UDT group, and bilateral UDT group. However, the median DFI and the percentage of men having a DFI above 30% were remarkably higher in the bilateral congenital group than in the other groups. The bilateral acquired group had the second highest number of men with a DFI >30%. No statistically significant differences were observed when unilateral UDT was compared with bilateral UDT in the congenital as well as the acquired UDT group.

Congenital UDT and acquired UDT vs. control group (Table 2)

No statistically significant differences were found in median DFI levels between the UDT groups and the control group. When taking the threshold of 30% into account men with congenital UDT (unilateral and bilateral UDT together) significantly more often had a DFI >30% (9 out 50 (18%)) compared with none in the control group (*p*-value 0.049). This also applies for

men with bilateral congenital UDT compared with controls (3 out 7 (43%)) vs. 0 out 22; *p*-value 0.01). For all other analyses no significant differences were found.

Congenital UDT: influence of age at orchiopexy on DFI (Table 3)

In the unilateral group, in total 19 men were treated with orchiopexy on or before the age of 24 months, 9 before the age of 18 months, and 7 before the age of 12 months. In the bilateral group 2 men were surgically treated before 24 months of age of whom one before the age of 18 months. Age at orchiopexy did not have a significant influence on the level of sperm DNA damage (median DFI as well as percentage of men with DFI> 30%) when using a cut off age at surgery of 24 months (Table 3), or 18 months or 12 months in both the unilateral as well as the bilateral group (data not shown).

Acquired UDT: influence of spontaneous descent or orchiopexy on DFI (Table 3)

In the unilateral group in 18 out the 38 men (47%) testicular descent was successfully awaited. Orchiopexy was needed in 20 men. In the bilateral group 6 out of 11 men (55%) had a spontaneous descent. No significant differences in median DFI or percentage of DFI >30% could be detected between men who had had a spontaneous descent in comparison with men needing orchiopexy in both the unilateral as well as the bilateral group.

Paternity: Effect of sperm DNA damage on the success rate of men attempting fatherhood

23 out of 99 men attempted fatherhood of whom 14 (61%) succeeded. There was no significant difference in DFI levels in men who succeeded in attempting fatherhood and the ones who were unsuccessful (median (range): men succeeding 14.2 (6.8 – 58.9) vs unsuccessful men 14.1 (1.5 – 35.9); *p*-value 0.69).

Logistic regression analyses

The type of UDT (congenital or acquired) did not correlate with a DFI above or below 30%. Univariate logistic regression analyses showed that FSH (IU/l) and days of ejaculatory abstinence had a positive correlation with DFI. The higher the FSH (IU/l) and the longer the ejaculatory abstinence the higher the odds of having a DFI above 30%. Motility was negatively correlated with DFI, the lower the number of progressive sperm the higher odds of having a DFI above 30%. When using multivariate logistic regression analyses only FSH (IU/l) remained statistically significant (Odds ratio (95% confidence interval) 1.12 (1.0064 – 1.2357) *p*-value 0.04).

Discussion

In literature, higher levels of sperm DNA damage are found in patients with a history UDT [25,29-31]. Most articles report sperm DNA fragmentation in men with a history of UDT visiting fertility clinics. The participants of the present study were from historical cohorts with UDT and therefore we feel that this is of great advantage because this study group reflects normal UDT population better. Moreover, our study is the first to report fertility assessed by SCSA in men with acquired UDT.

In our previous study no differences in fertility potential were found between congenital UDT and acquired UDT using 'traditional' fertility parameters (testicular volume, endocrinological evaluation and semen parameters) However, men who earlier had bilateral UDT (congenital or acquired) had the lowest fertility potential [8]. In that study a shared aetiology of congenital and acquired UDT was suggested. We hypothesized that UDT is a spectrum encompassing congenital UDT and acquired UDT while bilateral UDT is more severely affected than unilateral UDT.

In the present study the DFI was used as a reflection of the severity of spermatogenic failure [25,29-31]. If congenital and acquired UDT share the same aetiology little differences are expected in the level of sperm DNA damage between congenital and acquired UDT as was the case in our study. Also, one would expect the highest level of DFI in the bilateral groups. In this study, bilateral UDT especially bilateral congenital UDT seem more severely affected than unilateral UDT. Men with a history of bilateral congenital UDT had the highest median DFI and the highest percentage of DFI > 30%, followed by bilateral acquired UDT for a DFI above 30%, albeit all without significance. This might implicate that within the spectrum of UDT acquired and congenital UDT are not equally affected. Different assumptions on the differences between acquired and congenital UDT are made in the literature. On the one hand fewer fertility problems are assumed in acquired UDT since the crucial development of type A spermatogonia has probably already occurred during the first year of life before the testis

ascended [32]. On the other hand a study by Garcia found a mean decreased tubular fertility index value in men with acquired UDT, indicating impaired spermatogenesis similar to that in congenital UDT [33]. As mentioned before, the present study suggests a shared aetiology in congenital and acquired UDT, which is also suggested in two other studies [34,35].

In our previous studies, fertility was compromised in both the congenital group as well as the acquired group in comparison with a healthy control group [8,14]. In this study, all UDT groups had more often a DFI above 30% compared with the control group. However this only reached significance for the complete congenital UDT and bilateral congenital UDT group. Smith et al. reported on DFI measured by SCSA in men with UDT in comparison with a normospermic control group [29]. They also found that the DFI was significantly higher in the UDT group. The mean DFI measured in their patients with UDT was higher than in our participants. This could be due to the fact that all men included in their study had fertility problems attending a fertility clinic. The normospermic control group of that study had lower DFI levels compared to controls of our study. Our control group were men with proven fertility opting for vasectomy; their semen analyses, however, were unknown. A study by Smit et al., showed the same results; the DFI was significantly higher in patients with UDT in comparison with the control group [25]. Again the DFI in these patients was higher compared with our study, which again could be explained by the fact that the men in the study by Smit et al. were men with fertility problems attending an andrology outpatient clinic.

The influence of early orchiopexy on fertility in congenital UDT is still debated. While some studies suggest that orchiopexy at younger age results in better semen parameters [36-38] others, including ours, show no favourable effect of early treatment on semen characteristics [14,39-42]. High levels of sperm DNA damage found in humans with UDT can be explained by primary testicular dysfunction and/or high levels of reactive oxygen species (ROS) [29]. ROS are harmful for cells because they damage proteins, lipids, and DNA and have been associated with various diseases such as cancer, cardiovascular diseases, and diabetes [43]. ROS in testes induce apoptosis, and accelerate apoptotic cell death [44,45]. In rats, UDT is associated with ROS caused by hyperthermia and lower intratesticular testosterone levels [44-47]. It is unknown whether reducing the time of hyperthermia during childhood has an impact on sperm DNA damage in adult life. In case of congenital UDT, age at orchiopexy is a surrogate for the time of hyperthermia in the non-scrotal position. In our congenital UDT group, age at orchiopexy, did not have a significant effect on the amount of DNA damage.

In the acquired group, median DFI and the number of men with a DFI > 30% did not differ between men whose testis descended spontaneously and men who needed orchiopexy. It seems that the eventual outcome of the 'wait-and-see'-protocol does not lead to different fertility potential in adulthood. The level of sperm DNA damage remains unknown if men had

had immediate orchiopexy at diagnosis. However, we do not expect a great influence from immediate surgery on the amount of sperm DNA damage since other fertility parameters are not significantly different between men with immediate orchiopexy at diagnosis and men for who spontaneous descent was awaited [48].

Men from infertile couples and normal semen parameters more often have high levels of sperm DNA fragmentation [49,50]. In our study, 23 out of 99 men attempted fatherhood of whom 14 men succeeded. However, we found no difference in DFI between men who attempted fatherhood and succeeded compared with men who were unsuccessful. This is possible due to the low numbers of men attempting fatherhood.

This study has some limitations. The numbers in our study are relatively small, especially bilateral UDT, and due to logistical problems not all patients had a SCSA evaluation. The men from the historical cohorts included in this study were not part of randomized control trials. In the congenital group relatively few men were operated before the age of two years. Despite this we think this study adds to the current literature for the following reasons: [i] men included in this study are participants from historical cohorts and better reflect the normal UDT population than studies including men with UDT attending fertility clinics, [ii] it reports on a group of patients with congenital and acquired UDT instead of a group with UDT in whom previous testis position has not been taken into account, [iii] uni- and bilateral UDT are considered separately.

In conclusion, firstly, no significant differences between congenital and acquired UDT were found. Secondly, in comparison with a control group a DFI >30% was significantly more often found in the complete (i.e. unilateral and bilateral combined) congenital and bilateral congenital group. Furthermore, we could not demonstrate that age at orchiopexy in congenital UDT has an influence on sperm DNA damage. Finally, the outcome of the 'wait and see'-protocol for acquired UDT did not influence the eventual level of sperm DNA fragmentation.

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Part IV

Anomalies found by physical examination and scrotal ultrasound

Chapter 9

Scrotal ultrasound findings in previously congenital and acquired unilateral undescended testes and their contralateral normally descended testis.

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Summary

The aim of this study was to report on different anomalies found by physical examination and scrotal ultrasound in men with previously unilateral congenital undescended testes (UDT; N=50), acquired UDT (N=49), their contralateral normally descended testis (CNDT), and control testes (N=53).

Acquired UDT significantly more often had a testicular volume being <15ml than congenital UDT (88% vs. 68%). In the congenital group, significant differences were found between UDT and CNDT for soft consistency (UDT 36% vs. CNDT 14%), epididymal diameter (UDT 7.6mm vs. CNDT 8.9mm), testicular volume (UDT 9.8ml vs. CNDT 13.8ml), and inhomogeneous parenchyma (UDT 38% vs. CNDT 14%). In the acquired group, significant differences were found between UDT and CNDT for epididymal diameter (UDT 7.5mm vs. CNDT 8mm), testicular volume (UDT 9.3ml vs. CNDT 14.1ml), testicular volume <15ml (UDT 88% vs. CNDT 59%), and inhomogeneous parenchyma (UDT 27% vs. CNDT 6%). The following parameters of congenital UDT, acquired UDT, congenital CNDT, and/or acquired CNDT significantly differed compared with controls: soft testicular consistency (congenital UDT 36%, acquired UDT 20%, congenital CNDT 14%, acquired CNDT 12% vs. controls 0%), epididymal diameter (congenital UDT 7.6mm, acquired UDT 7.5mm, acquired CNDT 8mm vs. controls 9.2mm), testicular volume (congenital UDT 9.8ml, acquired UDT 9.3ml, congenital CNDT 13.8ml, acquired CNDT 14.1ml vs. control testes 15.8ml), testicular volume <15ml (congenital UDT 68%, acquired UDT 88%, congenital CNDT 66% vs. controls 43%), inhomogeneous parenchyma (congenital UDT 38%, acquired UDT 27%, congenital CNDT 14% vs. controls 0%), and testicular microlithiasis (congenital CNDT 24% vs. control testes 8%).

Few differences between congenital and acquired unilateral UDT and congenital and acquired CNDT support the hypothesis of a spectrum of maldescended testes containing congenital and acquired UDT instead of them being two different entities. The CNDT also has anomalies albeit less severe than the UDT, indicating that in unilateral UDT both testes are affected.

Introduction

Undescended testes (UDT) are a frequent urogenital abnormality that may be present at birth (congenital form) or develop later in life (acquired form) [1,2]. The congenital form never reaches a stable scrotal position after birth; and has an incidence around 1% at 1 year of age [3]. Moreover, 1-3% of boys develop an acquired UDT during childhood [4,5]. Acquired UDT is defined as a palpable UDT that previously had a normal scrotal position. Spontaneous descent during follow-up of children with acquired UDT is expected in 57-71% of patients [6,7]. UDT is associated with impaired fertility and testicular malignancies.

Physical examination and scrotal ultrasound is often performed in men with fertility problems, testicular abnormalities, paratesticular and testicular swellings, acute and chronic scrotal pain, and scrotal trauma [8]. Anomalies of the testes observed in patients with UDT can be caused by the primary condition, but also by testicular damage during orchiopexy [9]. Scrotal ultrasound as a diagnostic modality is widely used, but reference values in healthy adult men are scarce; there is no standardization and different formulas are used, for example to calculate testicular volume.

In our previous study we evaluated more traditional fertility parameters (e.g. testicular volume, endocrinological factors, and semen parameters) [10]. In this study, we more extensively describe anomalies found by physical examination and scrotal ultrasound (e.g. epididymal diameter (mm), presence of epididymal cyst(s), testicular volume, inhomogeneous parenchyma, presence of testicular microlithiasis (TM) and testicular cyst(s)). The anomalies described in this study can be associated with fertility problems, but also with the testicular dysgenesis syndrome which is associated with UDT [11]. We describe these anomalies for the UDT, their contralateral normally descended testis (CNDT) and a healthy control group. Most of the anomalies described in this study as well as all the results of the CNDT were not reported previously.

First, we evaluated the differences between congenital and acquired UDT. Fertility parameters between congenital and acquired UDT were not found to be significantly different in a previous study [10]. Therefore, we don't expect to find great differences for the prevalence of ultrasound anomalies between the two groups either, which would further support our hypothesis that congenital and acquired UDT are part of a spectrum of UDT instead of two different entities.

Second, we compared the CNDT with their UDT and a control group which could further enlighten whether scrotal anomalies in patients with UDT are affecting both testes or that it is an isolated problem of the UDT. Furthermore, ultrasound observations were compared between men with previously acquired UDT who had had a spontaneous descent and men with previously acquired UDT who had an orchiopexy.

Patients

Patients and controls were retrospectively included and data on physical examination and ultrasound were prospectively collected between 2005 and 2010.

Men included in the two patient groups described below all participated in studies evaluating fertility potential after treatment of congenital or acquired UDT performed at the Erasmus Medical Center. Inclusion and exclusion criteria of participants in those studies were described previously [10,12]. For this study we only selected men with a history of unilateral UDT and who had both testes at follow-up. In short:

The congenital UDT group (N=50) consisted of men treated for unilateral congenital UDT during childhood at various ages. Median = age at orchiopexy was 2.6 years (range = 0.1 - 10.3 years).

The acquired UDT group (N=49) consisted of men with previously acquired unilateral UDT who had annual follow-up examination until puberty [10]. For acquired UDT this implied that a previous normal stable scrotal position was documented at least once at birth and/or at follow-up either by the Regional Youth Health Care Institution, general practitioner, or hospital. Furthermore, at inclusion the testis had to be palpable without ipsilateral, inguinal, clinically present pathological findings or previous ipsilateral, inguinal surgery, and the most caudal testicular position after manipulation had to be non-scrotal or unstable scrotal position. Spontaneous descent was awaited until at least Tanner stage P2G2 and followed by orchiopexy in case of non-descent. Spontaneous descent had occurred in 24 participants. Median = age at orchiopexy in 25 participants was 13.3 years (range = 4.8 - 17.8 years). Reasons for earlier operation were inguinal complaints, a clinically present ipsilateral inguinal hernia or a completely immobile testis in the inguinal area.

Men from the control group (N=53) previously participated in a study evaluating fatherhood after use or non-use of sex steroid treatment during adolescence to reduce final height. Only those who had not received steroid treatment, had no history of UDT, and had no history of oncological treatment were included in this control group. These men had the same physical examination, and ultrasonic evaluation at our department as the men with previous congenital or acquired UDT [13]. For the control group mean values of both testes in one participant were used.

Methods

Physical examination and ultrasound

Consistency of the testis was examined with physical examination and was classified as normal or soft consistency. Scrotal ultrasound was performed in all men according to a standardised procedure using a high-resolution duplex echo machine (the Toshiba Nemio model SSA-550A) with a transducer frequency ranging from 7.5-12 MHz. The following parameters were registered: epididymal diameter (mm), presence of epididymal cyst(s), testicular volume, inhomogeneous parenchyma, and presence of TM, and testicular cyst(s).

For the measurement epididymal diameter, the diameter of the epididymal head in longitudinal direction was measured and a diameter above 12.0 mm was considered abnormal [14,15]. Epididymal cyst(s) were defined as sharply anechoic round lesions in the epididymis. Testicular volume was measured with an automated formula from the ultrasound machine; testicular volume = width x height x depth x 10^{-3} x 0.523* (*= 4/3π x ½ x ½ x ½). A volume of 15.0 ml or higher was considered normal, based on the cut-off value used in the guidelines on male infertility from the European Association of Urology and in our department [16]. Inhomogeneous parenchyma was defined as heterogeneous parenchyma with hypoechogenic and/or hyperechogenic areas within the testis. TM were hyperechogenic spots in the range = 2-3 mm without shadowing as previously defined [17]. Total number of spots in the entire testis was counted. Physical examination as well as the scrotal ultrasound was performed by two experienced physicians.

Statistical analysis

The testes of participants were categorised into three categories: UDT of congenital or acquired origin, CNDT of congenital or acquired origin, and control testes.

Differences in frequency of the anomalies found with physical examination and scrotal ultrasound between the different categories were analysed. First, we analysed the differences between congenital UDT and acquired UDT, and between congenital CNDT and acquired CNDT. Second, we evaluated the differences between the control group and all the patient groups (e.g. control group vs. congenital UDT, congenital CNDT, acquired UDT, and acquired CNDT). Subsequently the differences between UDT and CNDT for the congenital and the acquired group were analysed. Lastly, we analysed the differences between acquired UDT who had had spontaneously descended vs. acquired UDT that needed orchiopexy. Continuous variables were tested with the T-test; categorical variables with Chi squared test. When relevant the Fisher's exact test was used. For all analyses a two-sided *p*-value of < 0.05 was considered statistically significant.

Ethics

The study was reviewed and approved by the Medical Ethical Review Committee at Erasmus University Medical Centre in Rotterdam (MEC number 2004-206). Written informed consent was obtained from all patients who agreed to participate in the study.

Results

Congenital UDT vs. Acquired UDT (Table 1)

Men in the congenital group significantly more often had a testicular volume above 15ml (p = 0.02). No other statistically significant differences were found when comparing the frequency of anomalies found by physical examination and scrotal ultrasound between congenital and acquired UDT.

Congenital CNDT vs. Acquired CNDT (Table 1)

No statistically significant differences were found when comparing the frequency of anomalies found by physical examination and scrotal ultrasound between congenital and acquired CNDT.

Congenital group; UDT vs. CNDT (Table 2)

Congenital UDT in comparison with their CNDT had significantly more often a soft consistency (p = 0.01), smaller epididymal diameter (p = 0.004), lower testicular volume (p = 0.01), and more often inhomogeneous parenchyma (p = 0.006).

Acquired group; UDT vs. CNDT (Table 2)

Acquired UDT in comparison with their CNDT more often had a smaller epididymal diameter (p = 0.03), lower testicular volume (p <0.001), a testicular volume <15ml (p = 0.001), and inhomogeneous parenchyma (p = 0.006).

Congenital UDT and Acquired UDT vs. Control testes (Table 1)

Congenital or acquired UDT in comparison with control testes had significantly more often soft testicular consistency (congenital UDT vs. control testes p-value <0.001, acquired UDT vs. control testes $p = 0.001$), smaller epididymal diameter ($p < 0.001$), lower testicular volume ($p < 0.001$), more often a testicular volume <15ml (congenital UDT vs. control testes p = 0.01, acquired UDT vs. control testes p <0.001), and inhomogeneous parenchyma ($p < 0.001$). Acquired UDT more often had epididymal cyst(s) in comparison with the control testes ($p = 0.02$).

CNDT from the Congenital and CNDT from the Acquired group vs. Control testes (Table 1)

CNDT from both patient groups in comparison with control testes had significantly more often soft testicular consistency (congenital CNDT vs. control testes $p = 0.005$, acquired CNDT vs. control testes $p = 0.009$), epididymal cyst(s) (congenital CNDT vs. control testes $p = 0.003$, acquired CNDT vs. control testes $p = 0.01$), lower testicular volume (CNDT from the congenital group vs. control testes $p = 0.003$ and CNDT from the acquired group vs. control testes $p = 0.03$), and less often testicular cyst(s) (congenital CNDT vs. control testes $p = 0.01$, acquired CNDT vs. control testes $p = 0.02$).

CNDT from the congenital group more often had a testicular volume <15ml ($p = 0.02$), inhomogeneous parenchyma ($p = 0.005$) and TM in comparison with control testes ($p = 0.02$). CNDT from the acquired group had a smaller epididymal diameter than control testes ($p = 0.03$).

Acquired group; Spontaneous descended UDT vs. UDT which needed orchiopexy

Acquired UDT which descended spontaneously had significantly more often a larger epididymal diameter (median = (range =) spontaneous descend 8.3 mm (2.8 – 13.4) vs. orchiopexy 6.8 mm (3.1 – 9), $p = 0.01$). For the other anomalies no significant differences were found (data not shown).

Discussion

To our knowledge, this is the first study describing results of physical examination and ultrasound in men with previously congenital and acquired UDT, in comparison with the CNDT and a control group with normally descended testes. In this study, no statistically significant differences between congenital and acquired UDT were found, except for more often a testicular volume above 15ml in the congenital group. No differences were found between congenital CNDT and acquired CNDT. UDT in comparison with CNDT more often had a soft consistency (only for the congenital group), smaller epididymal diameter, lower testicular volume, a testicular volume <15ml (only for the acquired group), and inhomogeneous parenchyma. Overall, UDT and CNDT from the congenital and acquired groups in comparison with control testes more often had a soft testicular consistency, smaller epididymal diameter, more often epididymal cyst(s), a lower testicular volume, more often a testicular volume <15ml, and inhomogeneous parenchyma.

Abnormal testicular consistency in undescended testis is thought to be caused by histological changes (e.g. reduction of number of cells or seminiferous tubules and subsequent loss of testicular mass) [18]. In our study we more often found abnormal testicular consistency in congenital UDT in comparison with congenital CNDT and in UDT and CNDT in comparison with controls. Testicular consistency remains a subjective parameter and we did not use scales to standardize our findings.

An increased epididymal diameter can indicate an obstruction. In this study the median = epididymal diameter of all groups was below 9 mm which is normal considering the cut-off point of 12 mm used in the literature [14,15]. Epididymal diameter of the UDT of both patient groups and acquired CNDT was significantly smaller than controls. However, the question remains whether a significant difference in epididymal diameter but below 12 mm is clinically relevant.

In our study we found an incidence between 8 and 20% of epididymal cyst(s) in the different UDT and CNDT groups. In our control group only 2% of the men had epididymal cyst(s). A study comparing men with normally descended testes and men with undescended testes found 5% and 9% epididymal cyst (s) respectively [9]. Another study reported an incidence of epididymal cyst(s) between 2.8% and 7.6% in randomly selected men, including men with a history of maldescent, inguinal hernia, torsion of the testicle, and scrotal infections [19]. The clinical significance of epididymal cyst(s) remains unclear. It occurs more often in men with obstructive azoospermia [20]. However, the presence of bilateral epididymal cyst(s) is not always associated with azoospermia [21].

Median = testicular volume of our control group was 15.8ml (range = 8.2 - 27). This was significantly higher than that for the UDT and CNDT in both patient groups. Lenz et al., also found a smaller testicular volume in UDT (median = 10.5 ml, range = 3.9 – 17.1) in comparison with healthy controls (median = 14.1ml, range = 6.0 – 31.8) [19]. As both UDT and CNDT are smaller, a maldevelopment in both testes rather than an isolated problem in UDT can be suggested. Huff et al. and Zivkovic et al. found histological changes in contralateral normally descended testes which would support this hypothesis. They found that gonocytes failed to disappear and adult dark spermatogonia failed to appear in the UDT and in lesser extent in the CNDT [22,23]. In our study, UDT were significantly smaller than CNDT in both patient groups. This is in line with other studies reporting on differences in testicular volume between previously undescended testis and their CNDT [9,24,25]. Thus, UDT and CNDT are both smaller than the control group and complementary to this is the fact that UDT are also significantly smaller in comparison with the CNDT. Two possible explanations for this finding can be suggested. First, damage directly occurs because of the underlying condition in both testes instead of damage of just the UDT. Histopathological findings demonstrated that the UDT is more affected than its CNDT [22]. That might explain the even smaller volume of the UDT in comparison with the CNDT. Second, the smaller UDT in comparison to the CNDT might be caused by possible trauma during surgery. However, testicular volume did not significantly differ between men with previous acquired UDT who underwent orchiopexy and those with UDT that descended spontaneously, in line with another study [26]. We could not find an effect of surgery on testicular volume which is assumed in a study by Taskinen et al. They found that testicular volume was smaller in patients with no testicular artery detected by ultrasound, which the authors ascribed to damage owing to the operation [9]. In recent literature the cut-off value for testicular volume is debated. In different studies different methods to determine testicular volume are used. Also when ultrasound is used different formulas are used to calculate testicular volume. Meij-de Vries et al. studied the literature for normative values of testicular volume measured by ultrasound in healthy men. They concluded, after recalculating every outcome with the same formula, that the testicular volume cut-off value should be at 13,5ml [24]. Despite this, we decided to use the cut-off value of 15ml conform the guidelines on male infertility by the European Association of Urology [16].

A post-pubertal testis normally has a homogeneous texture of medium echogenicity [14]. In our group none of the control testes were inhomogeneous. One study in a general population found that 4.1% of 444 men from the general population had a very irregular pattern or TM [19]. The incidence of inhomogeneous parenchyma in UDT in the literature varies from 0 to 36% [9,19,27-29]. Age at evaluation in these studies is different. It is possible that inhomogeneous parenchyma develops at later age, because in our study the incidence of inhomogeneous parenchyma was between 6.1 and 38%. Comparison of studies is complicated by the fact that inhomogeneous parenchyma is a subjective parameter.

Testicular microlithiasis is associated with increased risk of the condition called carcinoma in situ (CIS) in men who also have risk factors for testicular cancer [30]. Most testicular germ cell tumours have CIS as precursor cell [31]. In our study the presence of even one single TM counted. Two studies reporting on TM in asymptomatic men and counting every TM found prevalences of 3 and 17.8% [29,32]. Seven-and-a-half percent of our control group had TM. The incidence of TM in men with a history of UDT varies from 3 to 8%, and even up to 42% in men with a history of UDT referred for subfertility [9,25,28-30]. However, in these studies TM was defined differently, varying from counting every TM to counting from >5 TM or it was not described. Both patient groups, UDT as well as CNDT, had significantly more often TM in comparison with controls. The only other study on TM and the two different types of UDT did not find a difference in prevalence of TM between boys with a history of congenital or acquired UDT, conform our results [33].

In our study two experienced physicians specialized in andrology performed physical examination and the ultrasound. This could influence our data, especially subjective parameters such as testicular consistency or homogenous parenchyma, because of inter observer variation. However, a previous study showed a good correlation between three investigators when measuring testicular volume by ultrasound [34].

In this study, the total numbers of testes are relatively small, therefore strong conclusions are hampered. We believe that despite small numbers this study adds important information about differences between congenital and acquired UDT as our study has certain strengths. First, our patients were part of historical cohorts instead of patients with UDT visiting fertility clinics, therefore patient selection by analysing only men with fertility problems does not play a part in these analyses. Second, the history of the type of UDT is known. In the literature mostly acquired UDT is not recognised as a distinct anomaly or history of testis position at birth is unknown.

Our study has a fairly low inclusion rate (28% for the congenital UDT group and 31% for the acquired UDT group). The inclusion rate in other studies regarding UDT varied between 29 and 73% [27,35-39]. A possible explanation for our low inclusion rate is the long interval between inclusion in the historical cohort and the request for evaluation of fertility parameters. Thereby, in our study patients had no regular check-ups in this interval. Patients might be more motivated to participate when the treatment is more recent or when follow-up is more frequent.

The patient groups are heterogeneous, meaning for congenital UDT the range of age at orchiopexy is wide and for the acquired UDT group half of them had a spontaneous descent and the other half needed orchiopexy. At inclusion of the congenital group maximum effort was taken to verify previous testis position, and we are convinced that these boys have true

congenital UDT. Apparently it is clinical practice that congenital UDT can become obvious at later age. For the acquired group, the study design (e.g. awaiting spontaneous descent and perform orchiopexy in case of non-descent) is the reason for having heterogeneity. We performed extra analyses to analyse differences between men who had had orchiopexy and men which testis descended spontaneously. We were unable to find significant differences except for epididymal diameter which we not consider of clinical relevance.

In conclusion, we found anomalies by physical examination and scrotal ultrasound in men with previous congenital or acquired UDT, with significant differences in the occurrence of anomalies between UDT, CNDT, and controls. This study supports our earlier results regarding fertility potential in congenital UDT and acquired UDT. We suggested a shared aetiology between these groups instead of them being two different entities. The similar anomalies and minimal differences found in this study between congenital and acquired UDT further support this hypothesis. The finding that CNDT also had statistically significant anomalies, albeit less severe than UDT, in comparison with the controls indicates that UDT affects both testes and is not an isolated problem of UDT.

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Part V General discussion

General discussion

In this thesis we aimed to elucidate differences between congenital and acquired undescended testis (UDT). We found differences in regard to surgical findings, however less differences in fertility parameters. We therefore conclude that the condition 'undescended testis' is a spectrum that contains congenital and acquired UDT. We found little evidence that performing orchiopexy as early as possible has an effect on fertility parameters later in life for congenital as well as acquired UDT. Unfortunately it seems that we have little influence on fertility potential later in life.

In this chapter we will discuss our main findings in detail by means of answering different questions using the outcomes of our studies compared to (recent) literature. We will conclude with implications for clinical practice and future perspectives.

Congenital and acquired undescended testes; different conditions with respect to anatomy?

UDT is one of the most frequent urogenital abnormalities, and can be of congenital or acquired origin [1-3]. The problem with the literature is that acquired UDT was only recently accepted as a distinct condition and most studies do not discriminate between congenital and acquired UDT. This complicates the interpretation regarding aetiology, natural course and treatment. For the participants of our cohort studies, great effort was put into ascertaining previous testis position at the inclusion of the initial studies, some 30 years ago. For congenital UDT much is known on the aetiology, natural course, and treatment. However these matters are less clear for acquired UDT and are still debated [1,3,4]. Congenital UDT are not likely to descent after 12 months of age, preferably need treatment between 9 and 12 months of age and 18 months at the latest, and are associated with infertility [5-8]. Two Dutch studies from Eijsbouts et al. and Sijstermans et al included only acquired UDT and found that spontaneous descent at puberty occurred in 57-71% of patients [9,10]. Therefore a 'wait-and-see' policy seems justified, although long term effects on fertility were until now uninvestigated. Two studies, which made the distinction between congenital and acquired UDT, suggest a shared aetiology [11,12]. Tanyel found that in congenital UDT, obliteration of the processus vaginalis by apoptosis is impeded; moreover if this process of obliterating the processus vaginalis is more subtly disturbed but persists the testis can descend normally and ascent later in time, i.e.an acquired UDT [11]. Clarnette and Hutson suggested that in acquired UDT the aetiology of ascent is a persisting fibrous remnant of the processus vaginalis, possibly through the above mechanism, which prevents natural elongation of the spermatic cord during growth [12]. Indeed in our study, a wide open processus vaginalis was more often found in congenital UDT and a closed processus vaginalis in acquired UDT, which is in line with the above mentioned hypothesis that

congenital and acquired UDT share the same aetiology but the process of maldescend is partly different (**chapter 3**). Since acquired UDT have been in the scrotum before, it is reasonable to assume that these testes have less or less severe anomalies in comparison with congenital UDT. We found less epididymal anomalies in acquired UDT, which was in line with a study by Guven and Kogan [13]. It seems fair to say that acquired UDT are not a variant of normally descended testes since anomalies do occur. Congenital and acquired UDT share parts of the same maldevelopmental process regarding testicular descent since anomalies overlap. However, since the anomalies are less profound in acquired UDT we could speculate that congenital and acquired UDT are part of a spectrum from sever maldescend to less severe maldescend instead of being equally abnormal.

Another question is where acquired UDT which have descended spontaneously are within this spectrum. We do not have anatomical findings of acquired UDT with spontaneous descent since they were not operated. Therefore it is difficult to be sure which mechanism underlies spontaneous descent.

In conclusion:

Congenital UDT and acquired UDT are different conditions with respect to anatomy within a spectrum of UDT. In contrast to most studies, in our studies maximum effort was put into sorting out previous testicular position to determine UDT of congenital or acquired origin.

Undescended testes; maldevelopment of both testes or isolated problem of the undescended testis?

Nowadays, UDT are accepted to be part of the testicular dysgenesis syndrome (TDS). This syndrome may also result in impaired spermatogenesis, hypospadias and testicular cancer and is related to environmental factors such as exposure to endocrine disruptors in utero [14]. TDS is a developmental disorder, which hypothetically influences both testes in case of unilateral UDT. Studies from Huff et al. and Zivkovic et al., which strengthen this hypothesis show histological anomalies in the contralateral normally descended testis (CNDT) in comparison with normal descended testes from healthy controls [15,16]. Furthermore, histopathological changes in CNDT are found to be similar but less severe in comparison with UDT [15,16]. This is in concordance with our observation that in CNDT more anomalies are found with physical examination and ultrasound in comparison with healthy controls, albeit less severe than in UDT (**chapter 4** and **chapter 9**).

In conclusion:

In boys with unilateral UDT both testes are negatively affected. The CNDT is affected to a lesser extent than UDT.

Congenital undescended testes; what is the optimal timing of orchiopexy for later fertility?

Evidence at young age

The recommended timing of orchiopexy for congenital UDT has decreased over the years. The guidelines from the European Association of Urology (EAU) nowadays advise orchiopexy between 6-12 months of age or 18 months at the latest [17]. A Dutch consensus based guideline recommended to operate congenital UDT between 6 and 12 months [18]. The rational for decreasing the age at orchiopexy derives from histological studies [19]. During mini puberty, gonocytes (neonatal germ cells) transform into adult dark (Ad) spermatogonia which are believed to be essential for future fertility [20]. In UDT, this transformation is interrupted or delayed followed by gonocytes undergoing apoptosis leading to infertility [20]. Hutson et al. hypothesized that the high temperature in UDT causes not only impaired transformation but also prevents apoptosis of the non-maturing gonocytes [21]. Furthermore, at the age of 4 years transformation from Ad spermatogonia into primary spermatocytes is defect in UDT [15]. Cortes et al. showed that no germ cells at orchiopexy is associated with infertility and that the youngest patient without germ cells was 18 months of age [22]. Based on these observations the recommended age for orchiopexy gradually lowered under the age of 12 months [22].The reason that most guidelines advice to wait until 6 months of age is the spontaneous descent rate. Most testes descent within the first 3 months of age which can be explained by the period of high levels of gonadotropins and testosterone in the mini puberty [5,23,24]. However, according to Kollin et al., spontaneous descent can occur up to 1 year with 8% descending between 6-12 months of age [5]. According to Hutson, the recommended age of orchiopexy between 3-9 months of age is based on 4 assumptions; (1) germ cell loss begins at 3-6 months of age, (2) this loss is preventable by placing the testis at the correct temperature in the scrotum, (3) no spontaneous descent occurs after 3 months, and (4) early surgery is safe with the use of magnifying glasses and adequate training [25]. One can debate whether it is worthwhile waiting until 12 months of age to await spontaneous descent because a little part of orchiopexies can be omitted due to spontaneous decent. The main question is whether it is safe to wait with regard to future fertility. Possible evidence that no avoidable damage is done by awaiting spontaneous descent is that in a retrospective study by Bilius et al.no differences in the prevalence of histological proven risk of infertility between boys operated before or after 18 months of age were found, implying that UDT is a pre-existent developmental disorder of

the testis [26]. Furthermore in a study by Kollin et al., testes which descended spontaneously during the first year of life, had the same testicular volume in comparison with testes which had had orchiopexy at 9 months of age[5]. On the other hand, a randomized controlled trial by the same author found clear beneficial effect on testicular volume when operated at 9 months of age in comparison with orchiopexy at three years of age [27]. They concluded that this is hopeful for future spermatogenesis since testicular volume is an approximate measure for spermatogenic activity [27]. One could speculate whether the difference would be as profound when randomizing between 9 months of age and for example 12 months or 18 months of age and furthermore whether testicular volume at this age is a representative of the number of germ cells or fertility later in life. Although guidelines over the years advised orchiopexy at younger age, in practice age at surgery remains far above the nowadays recommended age of 6-12 months [28]. There are several possible reasons for delayed correction in literature. Firstly, general practitioners and primary care providers might still not be aware of the optimal age for surgical correction. In two studies from England and Germany only 50% of the general practitioners and 59% of primary care paediatricians thought the optimum age of for orchiopexy was 12 months of age or younger [28,29]. Secondly, in most studies acquired UDT are not excluded which could explain a great part of late orchiopexies [30]. Thirdly, orchiopexy can be technically challenging especially in a small infant. Some surgeons may be hesitant to operate before the age of 12 months. Although orchiopexy is advocated as a relative simple procedure, Carson et al. and Ein et al. found an overall complication rate of 12% ; containing post-operative infection, wound dehiscence, bleeding and testicular atrophy [31,32]. Factors named to possibly influence complication rates and/or chance of testicular atrophy are testicular position (e.g. intra-abdominal testes), operation at younger age, operations by a surgeon performing low numbers of orchiopexies, and patients with a remaining open processus vaginalis [31,33]. If we are to perform every orchiopexy before the age of 12 months significant changes in referral patterns are required to save time and have the infant visit a paediatric urologist/surgeon before the age of 6 months [34]. As long as a no strong positive effect of surgery before 6 months of age is found, surgery before that time should not be advocated because of the spontaneous descent rate in the first months of life, the anaesthesia risk and the increased risk of complications in younger children.

Evidence at adult age

UDT may lead to fertility problems later in life, especially in bilateral cases [6,35,36]. However, literature is not consistent on the effect of age at surgery on fertility potential in adulthood. The studies are hard to compare due to several reasons; (1) unclear whether groups contain only congenital UDT or also acquired UDT, (2) groups containing unilateral UDT, bilateral UDT

or both, (3) uncertainty about the possible inclusion of retractile testes, (4) different age at surgery since recommended age at surgery in guidelines changed over time, and (5) different therapeutic strategies. To our knowledge randomized control trials (RCT) regarding age at orchiopexy and outcome at adult age are lacking. A systematic review by Chan et al. found evidence for the greatest fertility potential when operated before the age of 12 months and a decreased chance for testicular malignancy when operated before 10-11 years [37]. However, the RCT used in this review is describing testicular volume during childhood and not adulthood [27]. While some studies suggest a positive effect of early orchiopexy on fertility parameters (testicular volume, hormonal levels, and semen parameters) later in life [38-40], others cannot find a favourable effect of early treatment [41-46]. We were unable to find an age-dependent effect of orchiopexy on fertility parameters including testicular volume, endocrinological data (luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, and inhibin B), semen analysis (concentration, motility, and morphology), and level of DNA damage (**chapter 4** and **chapter 8**). However, it should be taken into account that only 8 patients were operated before the age of 12 months. The only suggestion of an age related effect was found in unilateral UDT when evaluating Sertoli cell function, by using serum Anti-Müllarian hormone (AMH) levels. Sertoli cell function was better when operated before 24 months of age in comparison with men operated after 24 months of age (**chapter 7**). In untreated unilateral UDT normospermia rates are found up to 38-54%, which means that over a third of men with untreated unilateral UDT have normal semen concentrations [47,48]. In our study described in (**chapter 4**), successful attempts to fatherhood were lower in the unilateral group in comparison with the control group (55% vs. 86%), though not statistically significant. However, the numbers of men attempting fatherhood were small. Paternity rate, found by Lee and Coughlin showed no significant difference between men with previously unilateral UDT and a healthy control group [49]. It is possible that men with unilateral UDT have a lower fertility potential i.e. smaller testicular volume, abnormal hormonal values and lower semen parameters, but the same paternity rates as men with normally descended testes. Most likely men with lower fertility potential are still able to father children. The question is whether fertility potential in unilateral UDT can be positively influenced by treatment at early age. The problem with the evaluation of treatment of unilateral UDT is the contribution of the CNDT, which also has signs of maldevelopment (**chapter 9**). Since anomalies found in CNDT cannot be improved by surgical intervention since the testis is already in its right position, it seems that if early treatment is effective, this is only the case for bilateral cases as was found in a study by Varela-Cives et al [50]. In one study by Lee, paternity rate in men with previously bilateral UDT was 65.3% and this was significantly lower in comparison with men with previously unilateral UDT and a control group in which the paternity rate were 89.7% and 93.2% respectively [6]. We found less fertility potential in bilateral UDT in comparison with a control group. However, when comparing bilateral UDT with unilateral UDT, except for

significantly lower semen concentration and lower AMH levels in bilateral UDT, no significant differences were found. The low number of bilateral UDT in our study might explain the less explicit differences between uni- and bilateral UDT. There is no question about whether congenital UDT need orchiopexy, firstly after 12 months of age they are unlikely to descent, secondly surgery before puberty decreases the chance of testicular malignancy (see further paragraph), and lastly a growing testis in the groin region is very unpleasant, however, the question remains what the right timing would be.

In conclusion:

In literature, evidence is contradictory for an age-dependent effect of orchiopexy on fertility parameters in unilateral UDT. In our study no clear effect of age at orchiopexy for unilateral UDT was found. For bilateral UDT evidence in the literature is somewhat stronger that early intervention might positively influences fertility potential, however this could not be confirmed in this thesis possibly due to low numbers.

Acquired undescended testes; do opposing treatment modalities have different outcomes?

Acquired UDT are predicted to perform better in comparison with congenital UDT since the essential transformation into Ad spermatogonia already occurred before the ascent of the testis [25,51]. However, Rusnack et al. found similar histopathological changes in acquired UDT as are found in congenital UDT [52]. Furthermore Foresta et al. found that the total number of germ cells was diminished similarly in acquired UDT and CNDT [53]. Until now no studies were available with long-term follow-up results with regards to fertility potential in adulthood in acquired UDT. In our study (**chapter 5**) fertility parameters in acquired UDT were impaired in comparison with controls. Furthermore in our study regarding anomalies found by physical examination and scrotal ultrasound in acquired UDT (**chapter 9**) anomalies were found in the UDT, and to lesser extent in the CNDT, in comparison with control testes. Therefore, it is safe to say that acquired UDT are not a variant of normally descended testes with just a mechanical problem. For acquired UDT two treatment strategies are advocated: immediate surgery at diagnosis or a 'wait-and-see'-protocol. Since >50% of acquired UDT descend spontaneously, one could await spontaneous descent, which diminishes the number of orchiopexies. The question is, while trying to omit half of orchiopexies with the 'wait-and-see'-protocol, whether possible additional damage by being non-scrotal may enhance fertility problems later in life and if so whether this can be prevented by immediate surgery at diagnosis. Most (paediatric) surgeons and urologists hypothesize that in spite of the chance of spontaneous descent,

immediate surgical correction at diagnosis could have positive effects as is assumed for congenital UDT [7]. This is based on the hypothesis of negative influence of higher thermal environment [3,54,55]. According to Hutson, fertility in acquired UDT is somewhat impaired since higher temperature still negatively influences the testis between roughly 5 to 15 years of age [25]. In a consensus meeting immediate surgery was advised even though they acknowledged that many testes descent spontaneously. It was argued that spermatogenic and endocrine function and not anatomical position should be the primary aim for treatment [56]. So far, no evidence was available that fertility potential in adulthood is improved with immediate surgery. In our study immediate surgery at diagnosis compared with the 'wait-and-see'-protocol showed no beneficial effect on fertility potential of immediate surgery (**chapter 6**). Furthermore, men whose testes descended spontaneously had no significant different fertility potential compared with men who needed orchiopexy in the 'wait-and-see'-protocol (**chapter 5**). Therefore it seems that there is no clear superior treatment protocol. At least for unilateral UDT, surgery at diagnosis does not seem to influence future fertility. The UDT as well as the CNDT is anomalous in acquired UDT (**chapter 9**). The diminished fertility parameters we found in unilateral acquired UDT might be due to the development disorder in both testes. Therefore we speculate that surgery of the UDT cannot improve fertility parameters. In bilateral UDT, we found a possible trend in favour of operation at diagnosis in comparison with the 'wait-and-see'- group. Semen concentration and progressive motility was higher in the orchiopexy at diagnosis group however without statistical significance. Thereby numbers of bilateral UDT in our study are small. In regard to complication rates due to orchiopexy, one could speculate that the complication rate might be lower in acquired UDT than in congenital UDT as operation occurs at older age. Furthermore, if intra-abdominal testes indeed account for a higher complication rate this will be lower in acquired UDT since an intra-abdominal position does not occur in acquired UDT. Unfortunately, to our knowledge no studies are available regarding complications in acquired UDT. The Dutch guidelines advise to discuss both treatment options (e.g. immediate surgery and 'wait-and-see'-strategy) for acquired UDT with the patient and parents [18]. For unilateral UDT immediate surgery at diagnosis does not yield improved fertility later in life. For bilateral UDT, patients and parents should be informed that there is an unknown effect on future fertility when operating on immediately, but in approximately 50% no operation is needed. Further studies with more bilateral UDT patients are needed.

In conclusion:

Acquired UDT are not a variant of normally descended testes. For unilateral UDT none of the treatment modalities seem to influence future fertility potential. For bilateral UDT a possible advantage is found for surgery at diagnosis.

Congenital and acquired undescended testes; different conditions with respect to fertility?

Since the acknowledgement of acquired UDT has happened relatively recent, current long-term follow-up studies in adults with a history of UDT include a mixture of patients with both congenital and acquired UDT. A review from Sijsterman et al. found that only 6 out of 46 articles made the distinction between congenital and acquired UDT [57]. As stated in the former paragraph fewer fertility problems are assumed in acquired UDT but similar histopathological changes in comparison with congenital UDT are found. One could hypothesize that long-term follow-up results are similar between congenital and acquired UDT [52].We confirm this hypothesis, showing that there were no significant differences in traditional fertility parameters (LH, FSH, testosterone, inhibin B, semen concentration, motility and morphology) between men with AUDT and men with CUDT (**chapter 5**). This is line with a study from Gracia et al who found a mean decreased tubular fertility index value in men with acquired UDT, possible implying impaired spermatogenesis similar to that in congenital UDT [58]. Although no differences in traditional fertility parameters could be obtained between congenital and acquired UDT, when using sperm DNA damage as a measure for fertility potential more evidence of a spectrum of UDT was found (**chapter 8**). The complete congenital group and bilateral congenital group significantly more often had a higher DNA damage compared with healthy controls. This was not statistically different for the acquired UDT group. Although the bilateral acquired group had the second highest prevalence of a DNA fragmentation index > 30%. Furthermore, Leydig cell function, represented by non-SHBG bound testosterone levels, and Sertoli cell function, represented by AMH levels, were more divergent in bilateral congenital UDT in comparison with bilateral acquired UDT. This might argue for a spectrum in which acquired UDT has indeed less fertility problems than congenital UDT.

In conclusion:

Few differences were found between congenital and acquired UDT. Nevertheless, congenital and acquired UDT are different conditions within a spectrum in which bilateral congenital UDT, followed by bilateral acquired UDT, are mostly affected in their fertility potential.

Congenital and acquired undescended testes; different conditions with respect to risk factors for testicular malignancies?

Patients with a history of UDT have an increased risk of developing testicular cancer with a relative risk of 4.8 [59]. There is evidence that surgery before puberty can decrease the relative

risk, however the relative risk remains higher than in the normal population [60,61]. Reducing the chance of testicular cancer by timely surgical correction can be explained by the so-called 'position theory' in which the abnormal position causes a higher thermal environment of the testis which induces carcinogenetic changes [62]. The TDS, in which genetic and endocrine disruptors are related to undescended testes and testicular malignancies, would explain the inability of surgery to decrease the risk of testicular cancer comparable with the normal population[14,62]. There is a hypothesis why in acquired UDT the risk for testicular malignancy is not increased [21]. The high temperature in congenital UDT, during the 'mini puberty' around the age of 2-3 months, would not only cause impaired transformation of the gonocytes but also prevent apoptosis of non-maturing gonocytes [21]. These non-matured remaining gonocytes are hypothesized to be the source for carcinoma in situ (CIS) [63]. CIS is a precursor for testicular malignancy [64]. In acquired UDT, it is hypothesized that no non-mature gonocytes are present to be the source of CIS since the abnormal temperature occurs after the essential transformation of gonocytes [21]. Anomalies associated with CIS are inhomogeneous parenchyma and testicular microlithiasis [65,66]. We were unable to find differences in the presence of inhomogeneous parenchyma or testicular microlithiasis between congenital and acquired UDT as well as congenital and acquired CNDT (**chapter 9**). In our study population, only one patient in the congenital group had a history of testicular cancer in his CNDT. Our patients are relatively young because the peak incidence of testicular cancer is in the third and fourth decade of life [67]. It is possible that if we had a longer follow up more malignancies would have occurred.

In conclusion:

From literature it appears that acquired UDT have a lower chance of developing testicular malignancies than congenital UDT. In our study, we were unable to confirm this possible due to small sample sizes and short follow-up time. However no significant differences were found between congenital and acquired UDT with regard to ultrasound anomalies associated with CIS.

Strengths and limitations of this thesis

Strengths

Men participating in this thesis were part of a historical cohort. This group represents the overall 'UDT population' better than men with a history of UDT visiting fertility clinics. In most studies regarding fertility in adult men with previously UDT earlier testicular position during childhood is not mentioned. Without this information it is almost impossible to differentiate

between congenital and acquired UDT at adult age since the information from youth health care systems might be difficult to obtain many years later. The benefit of our historical cohort is that at primary inclusion of our patients in the initial studies during childhood, some 30 years ago, great effort was put into differentiating between congenital and acquired UDT [9,68]. Some say paternity is the best way to evaluate fertility but mostly paternity is not taken into account in studies evaluating fertility potential in men with previously UDT. In this thesis paternity was evaluated, but unfortunately, not many participants attempted fatherhood yet. We are fortunate to have a control group for comparison of fertility parameters, since in most studies regarding UDT and fertility potential a control group is lacking.

Limitations

The inclusion rate is relatively low in our studies. However, our inclusion rates are comparable to other long-term follow studies regarding men with a history of UDT. Specific motivations for participating can introduce a selection bias especially in fertility studies. One could speculate that for example suspected infertility or prolonged intend to fatherhood can be a reason for both participation and refusal. Maximum effort was taken to find out whether selection bias was to be expected for the participants. Men who did not respond or did not participate did not differ significantly from men participating regarding age at orchiopexy, uni- or bilateral UDT, and current age. All men who responded but did not want to participate received a questionnaire regarding attempting fatherhood, number of pregnancies, time to pregnancy, active child-wish, and duration of active child wish. The only difference found was observed in the congenital group where men who participated had a longer duration of child-wish. If there is a selection bias due to fertility problems there is no reason to suspect that this is different for our various patient groups (e.g. congenital UDT group, acquired UDT group or control group). Unfortunately there are relatively few patients with bilateral UDT included in our studies, especially since the evaluation of therapeutic effects is mostly needed for this group. When performing physical examinations and scrotal ultrasound for study purposes it is best to have little variation among the investigators. For this thesis, three experienced physicians performed the physical examinations and ultrasounds. This could influence the data, especially subjective parameters such as testicular consistency or homogenous parenchyma, because of inter observer variation. However, a previous study showed a good correlation between three investigators when measuring testicular volume by ultrasound [69]. Lastly, in our studies only one semen sample was available. However, semen quality has a great biological variation; therefore 2 or more samples would be preferred. On the other hand this biological variation is present in all our patient groups and a previous study has shown substantial agreement between consecutive semen analyses in young men [70].

Implications for clinical practice based on conclusions of this thesis

Congenital UDT

Congenital UDT are not expected to descend after 12 months of age and after this orchiopexy is mandatory. The question is what the best time for surgery is. The guidelines advice surgery for uni- and bilateral UDT between 6 and 12 months of age because of the possible positive effects on fertility potential later in life. In this thesis, unilateral UDT had worse fertility potential in comparison with healthy controls, however no effect of age at surgery was found. We think that surgery for unilateral UDT in the second year of life will not negatively influence fertility outcomes. We recommend early orchiopexy because of the possibility to decrease the change of testicular malignancies and complaints of a growing testis in the groin region. More importantly, orchiopexy must be performed by a dedicated (paediatric) surgeon or urologist in a hospital with optimal facilities for operating children. Parents should be counselled that in case of unilateral UDT the concerns for fertility problems are less severe than for bilateral UDT and furthermore that surgery will not influence fertility outcomes even if this correction is in the second year of life.

For bilateral UDT, we cannot draw the same conclusion. The negative effect of having bilateral UDT on fertility is unmistakable. In this thesis, we found the severest compromised fertility potential in bilateral UDT in comparison with a control group and unilateral UDT. We were unable to detect a positive effect of early surgery however this might be due to the small numbers. Parents should be counselled that despite surgery there still might be fertility problems later in life possibly not corrected by early intervention. However, with early surgery the boys get the biggest chance of a positive effect. Therefore, we advise to perform surgery between 6 and 12 months of age.

Acquired UDT

Since > 50% descent spontaneously and operation is not without risk we should carefully weigh the benefit against the complications of immediate surgical intervention for acquired UDT. In this thesis, we found impaired fertility potential in unilateral as well as bilateral acquired UDT in comparison with a control group. We were unable to detect a significant positive effect of immediate intervention at diagnosis. Also no harm was found for the 'wait-and-see' policy. This was the case for unilateral and bilateral UDT. The guidelines recommend a discussion with parents and patient concerning the possible positive effect of surgery at diagnosis and the chance of spontaneous descent. With our results kept in mind for unilateral acquired UDT a 'wait-and-see' policy seems safe and immediate surgery should only be performed if parents

and/or patient request this. Surgery will not influence fertility potential later in life and this should be elucidated. For bilateral UDT, we found a possible trend for early surgical intervention but our group was fairly small. Therefore, we feel that when counselling parents and/or patient in case of bilateral acquired UDT all treatment modalities should be discussed with a preference for immediate surgery.

Future perspectives

The evaluation of the true effect of early surgery of congenital UDT with respect of fertility later in life is problematic. The problem is that the follow-up timing between (early) intervention and measurement of the outcome is very long (20-30 years for fertility and 30-40 years for testicular malignancies). In most fertility studies published nowadays the median age of orchiopexy is relatively late, since early operation was not yet recommended. Also at that time, as mentioned before, there was no discrimination between congenital and acquired UDT, since this distinction is only made recently. Because patients, who have been treated after the change of the guidelines, will reach adulthood soon, follow-up studies in the near future will contain more patients who had early orchiopexy. Furthermore, hopefully more attention is paid to previous testicular position at the initial doctor's visit to diagnose acquired UDT, which might influence treatment and inclusion in potential follow-up studies.

Ideally, randomized controlled trials with different treatment modalities and follow-up from birth until adulthood, with primary outcomes paternity and malignancy, would be the best way to assess the optimal treatment for congenital and acquired UDT. The problem with these randomized controlled trials is that they require long follow-up up to 40 years. Also large numbers of participants are required to demonstrate differences since the differences expected for fertility might be subtle and malignancy is rare. Besides, the emphasis should be on bilateral UDT, which is less frequent than unilateral UDT. The realisation of such a trial in practice is very complex if not impossible.

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Part VI

Appendices

Summary

Undescended testis (UDT) is one of the most frequent urogenital abnormalities, and can be of congenital or acquired origin. The problem with most literature is that since acquired UDT was only recently accepted as a distinct entity, it does not discriminate between congenital and acquired UDT. For congenital UDT much is known on the aetiology, natural course, and treatment, however, these matters are less clear for acquired UDT and are still debated. Congenital UDT are not likely to descent spontaneously after 12 months of age and are associated with an increased risk of fertility problems and testicular malignancies. Over 50% of acquired UDT descend spontaneously before or during puberty. For acquired UDT the chance of fertility problems and risks for testicular malignancies are unknown. It is hypothesized that early treatment in congenital UDT can contribute to less problems in adulthood based on histological parameters in childhood, however this needs to be verified in adulthood. For acquired UDT there is no consensus on treatment protocols and although less fertility problems are expected than in congenital UDT, this needs to be confirmed. This thesis analyses outcomes after different treatments for congenital and acquired UDT and elucidates differences between congenital and acquired UDT regarding natural course, fertility, endocrinology and ultrasound anomalies. Men from the congenital group are participants from a historical cohort in which boys were operated upon at several ages during childhood. Men from the acquired group were men with a history of acquired UDT who, during childhood, were either followed annually until puberty to await spontaneous descent with orchiopexy in case of non-descent or were operated upon immediate after diagnosis. At inclusion of the initial studies great effort was put into ascertaining previous testis position to differentiate congenital from acquired UDT.

Part I – Surgical findings of acquired and congenital undescended testes

We tried to explain why some acquired UDT descend spontaneously while others need orchiopexy on the basis of anatomical findings. Anatomical findings during surgery, described in **chapter 2**, suggested that failure of elongation of the spermatic cord is hampered by a fibrous string in acquired UDT. Acquired UDT located at the external annulus have limited mobility and are unlikely to descend spontaneously. In contrast to this are testes lying in the superficial inguinal pouch which are more likely to be mobile pre operatively and therefore we hypothesize that they can descent spontaneously since growth of testes under the influence of

hormones at puberty might cause these testes to overcome the strength of the fibrous string. We assume that this is the explanation why some acquired UDT descend spontaneously while others do not. Unfortunately, we could not confirm this hypothesis since for obvious reasons we do not have anatomical findings from testes which descended spontaneously. In **chapter 3,** anatomical differences between congenital and acquired UDT were described. Most caudal testis position pre operatively in congenital UDT was at the inguinal region or at scrotal entrance, and at surgery they were found inside or just outside the inguinal canal. In contrast to this, acquired UDT could often be brought into an unstable scrotal position and at surgery they were most often found in the superficial inguinal pouch. Acquired UDT had fewer epididymal anomalies and more often a closed processus vaginalis. Congenital UDT more often had wide open processus vaginalis. These results indicate that congenital UDT and acquired UDT are two distinct entities.

Part II – Fertility potential in men with congenital UDT compared with acquired UDT. Analysing age at surgery for congenital UDT and evaluating different treatment protocols for acquired UDT

In the second part of this thesis regarding traditional fertility parameters (testicular volume, LH, FSH, testosterone, inhibin B, and semen analysis), the study in **chapter 4** describes that fertility potential in congenital UDT is compromised in comparison with healthy controls. We could not find evidence that age at orchiopexy (range 0.1 – 14.6 yrs) influenced fertility parameters. Men with bilateral congenital UDT had lower sperm concentration in comparison with unilateral congenital UDT. The study in **chapter 5,** showed that men with previously acquired UDT have a compromised fertility in comparison with a healthy control group but comparable with men with congenital UDT. This indicates that acquired UDT are not a variant of normally descended testes. In that study spontaneous descent was awaited until puberty followed by orchiopexy in case of non-descent (i.e. 'wait-and-see'-protocol). No significant differences between men who had had spontaneous descent in comparison with men who needed orchiopexy after the 'wait-and-see' protocol was found. The study in **chapter 6,** found no differences in fertility parameters when comparing men with acquired UDT who had had immediate treatment at diagnosis and men with acquired UDT who had the 'wait-and-see' protocol. Also age at orchiopexy, if operated upon, in acquired UDT had no influence on analysed semen parameters. It was thereby concluded that, unfortunately we have little influence on fertility potential later in life with our treatment.

Part III – Evaluation of endocrinological function and semen quality with less traditional methods

The study in **chapter 7,** evaluated Leydig cell function with Insulin-like peptide 3 (INSL3) and Sertoli cell function with Anti-Müllerian hormone (AMH) serum levels within and between the congenital and acquired UDT groups. Age at surgery in the congenital group and the different treatment protocols in the acquired UDT group were also evaluated. Leydig cell function evaluated by INSL3 showed impairment for congenital and acquired UDT but did not discriminate between the different UDT groups. No effect of age at surgery in the congenital group or the 'wait-and-see'-protocol in the acquired group on Leydig cell function was found. Sertoli cell function was most affected in the bilateral congenital group. Sertoli cell function evaluated by AMH showed an advantage for early operation in unilateral congenital UDT. Sertoli cell function was more negatively affected in congenital UDT in comparison with acquired UDT and in bilateral congenital UDT in comparison with unilateral congenital UDT. No effect on Sertoli cell function of the 'wait-and-see' protocol was found in acquired UDT. In the study described in **chapter 8,** sperm DNA damage was analysed using Sperm Chromatin Structure Assay (SCSA). We found no significant differences between congenital and acquired UDT. Furthermore, we could not find an effect of age at orchiopexy on the level of sperm DNA damage in men with congenital UDT. Also no significant differences between men with acquired UDT who had had spontaneous descent in comparison with men needing orchiopexy could be detected. The highest level of DNA damage was found in the bilateral congenital group.

Part IV – Anomalies found by physical examination and scrotal ultrasound

In the study described in **chapter 9,** we observed more anomalies by physical examination and scrotal ultrasound in men with previously congenital and acquired UDT in comparison with a control group. Congenital UDT were more often > 15ml in comparison with acquired UDT. No differences were found when comparing contralateral normally descended testes (CNDT) from the congenital group with those of the acquired group. CNDT also showed significant anomalies in comparison with a control group, albeit less severe in comparison with UDT in the congenital as well as the acquired group.

Part V- Discussion

In this thesis we were unable to find a significant effect of age at surgery on fertility parameters for congenital UDT. For unilateral congenital UDT we therefore think that surgery in the second year of life will not negatively influence outcomes. More importantly, we recommend that a dedicated surgeon or urologist performs the orchiopexy in a hospital with optimal facilities for operating children. We do recommend surgery at early age because congenital UDT are not expected to descent after 12 months of age, also the chance of testicular malignancies decreases when operated before puberty , and because of possible complaints of a growing testis in the groin. Parents should be counselled that the intervention will not influence fertility outcomes but that few fertility problems in unilateral congenital UDT are expected. For bilateral congenital UDT, we advise to perform surgery between 6 to 12 months of age even though we were unable to detect a significant positive effect of early surgery on fertility parameters, possibly due to low numbers. Parents should be counselled that despite surgery there still might be fertility problems later in life possibly not corrected by early intervention. However, with early surgery the boys get the biggest chance of a positive effect.

For acquired UDT, we found impaired fertility potential for unilateral and bilateral UDT in comparison with a control group but comparable to the congenital UDT group. For unilateral and bilateral UDT, we were unable to detect a positive effect of immediate intervention at diagnosis. Also no harm was found of the 'wait-and-see' policy. Thereby, the chance of a spontaneous descent is > 50 %. We therefore advice to discuss the different treatment strategies with patient and parents. For unilateral acquired UDT a 'wait-and-see' protocol seems safe and immediate intervention will not influence fertility potential. Orchiopexy at diagnosis should only be performed if patients/parents request this. For bilateral, a possible trend towards early intervention was found. Therefore, we feel that when counselling parents and/or patient all treatment modalities should be discussed but with a preference for immediate surgery.

Samenvatting

De niet ingedaalde testikel (NIT) (enkel- of dubbelzijdig) is een van de meest voorkomende urogenitale afwijkingen en kan van aangeboren of verworven origine zijn. Een aangeboren NIT is een testikel die nooit tot in de balzak (scrotum) is ingedaald. Een verworven NIT daarentegen heeft aanvankelijk een scrotale positie gehad maar heeft jaren later een positie boven het scrotum, in het liesgebied, ingenomen. In de de meeste literatuur wordt er geen onderscheid gemaakt tussen aangeboren en verworven NIT. Het is nog niet zo lang dat verworven NIT een aparte entiteit is. Over de etiologie, spontaan beloop en behandeling van aangeboren NIT is relatief veel bekend. Daarentegen is de verworven NIT veel minder bekend en nog steeds onderwerp van discussie. Aangeboren NIT dalen na de leeftijd van 12 maanden niet meer spontaan in en zijn geassocieerd met een verhoogd risico op fertiliteitproblemen en teelbalkanker. Meer dan 50% van verworven NIT dalen spontaan in voor of tijdens de puberteit. Over het risico van fertiliteitproblemen en teelbalkanker bij verworven NIT is weinig bekend. Behandeling van de aangeboren NIT is operatie op jonge leeftijd waarbij de testikel in de balzak wordt gefixeerd (orchidopexie). De hypothese dat behandeling van aangeboren NIT op zeer jonge leeftijd kan bijdragen aan minder problemen op volwassen leeftijd is gebaseerd op histologische studies bij kinderen, maar dit moet geverifieerd worden bij volwassen. Voor verworven NIT is er geen consensus over het te volgen behandelingsprotocol, spontane indaling afwachten tot de puberteit of operatie na stellen van de diagnose. Er worden minder fertiliteitproblemen verwacht bij verworven NIT echter dit moet bevestigd worden.

Dit proefschrift analyseert uitkomsten van verschillende behandelingen van aangeboren en verworven NIT bij volwassen mannen. Ook wordt er gekeken naar de verschillen tussen aangeboren en verworven NIT betreffende natuurlijk beloop, fertiliteitparameters, en echografische afwijkingen. Mannen in de aangeboren groep komen uit een historisch cohort waarin jongens op verschillende leeftijden tijdens de kindertijd geopereerd werden vanwege hun NIT. Mannen uit de verworven groep zijn mannen met een voorgeschiedenis van verworven NIT die op verschillende manieren behandeld werden tijdens hun kindertijd. Een deel werd jaarlijks gevolgd waarbij spontane indaling tot in de puberteit werd afgewacht. Zij ondergingen een operatie als de testikel niet spontaan indaalde (afwachtend-beleid). Een ander deel werd na het vaststellen van een verworven NIT direct geopereerd. Bij de primaire inclusie van beide groepen werd veel aandacht gegeven aan het achterhalen van eerder vastgestelde posities van de testikel op de kinderleeftijd. Op die manier kon zo goed mogelijk worden vastgesteld of de testikel een aangeboren of verworven NIT was.

Deel I- Chirurgische bevindingen bij aangeboren en verworven niet ingedaalde testikels

In dit proefschrift wordt vanuit de bij operatie gevonden anatomische afwijkingen getracht een verklaring te vinden waarom sommige verworven NIT spontaan indalen en voor andere een orchidopexie nodig is. Anatomische bevindingen tijdens de operatie van verworven NIT, beschreven in **hoofdstuk 2,** suggereren dat het onvermogen van het meegroeien en dus langer worden van de zaadstreng (funiculus spermaticus) wordt veroorzaakt door een fibreuze streng. Verworven NIT gelokaliseerd bij de annulus externus hebben beperkte bewegelijkheid en het is onwaarschijnlijk dat deze testikels spontaan zullen indalen. Dit in tegenstelling tot testikels die boven de uitgang van het lieskanaal liggen (superficial inguinal pouch) en meer mobiel zijn. De hypothese is dat deze testikels spontaan kunnen indalen doordat, door de groei van de testikels onder invloed van hormonen tijdens de puberteit, de zwaarder geworden testikels de weerstand van de fibreuze streng kunnen overwinnen. Het is aannemelijk dat dit een verklaring is waarom sommige verworven NIT spontaan indalen en andere een operatie nodig hebben. Helaas kan deze hypothese niet duidelijk bevestigd worden omdat er uiteraard geen anatomische bevindingen beschikbaar zijn van testikels die spontaan indaalden. In **hoofdstuk 3,** worden anatomische verschillen tussen aangeboren en verworven NIT beschreven. De laagste pre operatieve positie van een aangeboren NIT was in het liesgebied of bij de ingang van het scrotum. Tijdens de operatie werden aangeboren NIT gevonden in of net buiten het lieskanaal. Dit in tegenstelling tot verworven NIT, welke vaak voor de operatie in een instabiele scrotale positie gebracht konden worden. Verworven NIT werden tijdens de operatie vaak in de superficial inguinal pouch gevonden. Verworven NIT hadden minder bijbal (epididymis) afwijkingen en vaker een gesloten uitstulping van het buikvlies (processus vaginalis). Aangeboren NIT hadden vaker een wijd open processus vaginalis. Op basis van deze resultaten concluderen wij dat aangeboren en verworven NIT twee verschillende vormen van NIT zijn.

Deel II- Fertiliteitparameters bij mannen met aangeboren NIT in vergelijking met verworven NIT. Analyse van invloed van leeftijd bij operatie van aangeboren NIT en evaluatie van verschillende behandelingsprotocollen van verworven NIT

Het tweede deel van dit proefschrift beschrijft fertiliteitparameters (testikel volume, LH, FSH, testosteron, inhibine B en semenanalyse). In de studie beschreven in **hoofdstuk 4** worden minder goede fertiliteitparameters gevonden bij mannen met een voorgeschiedenis van aangeboren NIT in vergelijking met een controle groep. Wij konden geen relatie vinden tussen

de leeftijd bij operatie (range 0.1 – 14.6 jaar) en fertiliteitparameters. Mannen met dubbelzijdig aangeboren NIT hadden lagere semen concentraties in vergelijking met mannen met enkelzijdige aangeboren NIT. De studie beschreven in **hoofdstuk 5** liet zien dat mannen met een voorgeschiedenis van verworven NIT verminderde fertiliteitparameters hebben in vergelijking met een controle groep. Significante verschillen in fertiliteitparameters tussen aangeboren NIT en verworven NIT konden niet worden gevonden. Dit laat zien dat verworven NIT geen variant zijn van normaal ingedaalde testikels. Ook werden er geen significante verschillen gevonden tussen mannen met een spontane indaling en mannen die een orchidopexie moesten ondergaan. Mannen die als kind direct na het stellen van de diagnose verworven NIT een operatie ondergingen hadden ook geen significant betere fertiliteitparameters in vergelijk met de mannen bij wie een afwachtend beleid werd gevoerd (**hoofdstuk 6**). In de verworven groep had de leeftijd van operatief ingrijpen geen invloed op semenparameters. Geconcludeerd kan worden dat de verschillende behandelingsvormen op de kinderleeftijd weinig invloed lijken te hebben op fertiliteitparameters op volwassen leeftijd.

Deel III- Evaluatie van hormonale functie van de testikel en semen kwaliteit

De studie beschreven in **hoofdstuk 7,** evalueert Leydig cel functie met insuline-like peptide 3 (INSL3) en Sertoli cel functie met Anti-Müller hormoon (AMH). De verschillen tussen de twee NIT groepen werd geanalyseerd. Ook werd leeftijd bij operatie in de aangeboren groep en het effect van een afwachtend-beleid in de verworven groep geëvalueerd. De Leydig cel functie van zowel de aangeboren als verworven NIT is verminderd maar de verschillen zijn niet significant. Noch leeftijd bij operatie in de aangeboren NIT groep alsook de uitkomst van het afwachtend-beleid binnen de verworven NIT groep bleek invloed te hebben op de Leydig cel functie. Sertoli cel functie was het sterkst verminderd in de groep van dubbelzijdige aangeboren NIT. In de aangeboren NIT groep liet de Sertoli cel functie, geëvalueerd met AMH, een voordeel zien ten gunste van vroeg opereren. In vergelijking met de verworven NIT was de Sertoli cel functie van de aangeboren NIT het sterkst afwijkend. Hetzelfde werd gevonden t.a.v. dubbelzijdige aangeboren NIT in vergelijking met enkelzijdige aangeboren NIT. Het afwachtend-beleid in de verworven NIT groep liet geen negatief effect zien op Sertoli cel functie. In de studie beschreven in **hoofdstuk 8** werd DNA schade in semen geanalyseerd met behulp van Sperm Chromatin Structure Assay (SCSA). Er werd geen significant verschil gevonden tussen aangeboren en verworven NIT. Er werd geen effect van leeftijd van operatie gevonden op de mate van DNA schade in aangeboren NIT. Ook werd er in de verworven NIT groep geen significant verschil gevonden tussen mannen wiens testikels spontaan indaalden

versus mannen die een orchidopexie nodig hadden. De meeste DNA schade werd gevonden in het semen van mannen met dubbelzijdig aangeboren NIT.

Deel IV-Afwijkingen gevonden bij lichamelijk onderzoek en scrotale echografie

In de studie beschreven in **hoofdstuk 9**, werden bij lichamelijk onderzoek en scrotale echografie, in vergelijking met een controle groep, meer afwijkingen gevonden in aangeboren dan in de verworven NIT. Aangeboren NIT hadden vaker een volume boven de 15ml in vergelijking met verworven NIT. Er werd geen verschil gevonden tussen de contralaterale normaal ingedaalde testikel (CNIT) in de aangeboren groep in vergelijking met de CNIT van de verworven groep. De CNIT had echter significant meer afwijkingen in vergelijking met testikels uit de controle groep, maar wel minder ernstig dan de gevonden afwijkingen in de aangeboren alsook de verworven NIT groep.

Deel V- Discussie

In dit proefschrift werd bij aangeboren NIT geen duidelijke relatie tussen leeftijd bij operatie en fertiliteitsparameters op latere leeftijd aangetoond. Voor enkelzijdige aangeboren NIT is het niet waarschijnlijk dat een operatie voor het tweede levensjaar een positieve invloed zal hebben op latere fertiliteit. Belangrijker is dat de operatie uitgevoerd wordt door een betrokken, ervaren (kinder)chirurg of uroloog in een centrum met goede faciliteiten voor operaties bij kinderen. Het advies is wel om de operatie op jonge leeftijd uit te voeren omdat aangeboren NIT niet meer indalen na de leeftijd van 12 maanden, een operatie voor de puberteit de kans op teelbalkanker verkleint, en omdat operatie mogelijke klachten van een groeiende testikel in de lies regio in de puberteit voorkomt. Ouders moeten uitleg krijgen dat de operatie geen invloed heeft op fertiliteitparameters later maar dat er ook weinig problemen te verwachten zijn bij enkelzijdige NIT. Voor dubbelzijdige aangeboren NIT is het advies om de operatie te verrichten tussen 6 en 12 maanden, ondanks het feit dat wij geen duidelijk effect van leeftijd bij operatie hebben aangetoond (mogelijk veroorzaakt door kleine aantallen). Ouders moeten worden ingelicht dat ondanks operatie op jonge leeftijd er later nog steeds fertiliteitsproblemen kunnen zijn welke mogelijk niet worden beïnvloed door vroeg ingrijpen. Maar door vroeg opereren krijgen de jongens wel de grootste kans op een positief effect.

Bij verworven NIT werden, in vergelijking met een controle groep, slechtere fertiliteitparameters gevonden zowel bij enkelzijdige als dubbelzijdige NIT. Deze afwijkingen

komen overeen met de gevondenen afwijkingen in de aangeboren NIT groep. Voor zowel enkelzijdige als dubbelzijdige NIT werd geen positief effect gevonden van directe operatie bij diagnose. Ook vonden wij geen schadelijk effect van het afwachtend beleid. Omdat > 50% van verworven NIT spontaan kunnen indalen, is het advies om de verschillende behandel strategieën, afwachten tot de puberteit of operatie na stellen van de diagnose, met ouders en patiënt te bespreken. Bij enkelzijdig NIT lijken beide hiervoor genoemde behandelstrategieën niet bij te dragen aan betere fertiliteit op latere leeftijd. Orchidopexie bij diagnose zou alleen uitgevoerd moeten worden indien ouders/ patiënten dit wensen. Voor dubbelzijdige verworven NIT werd een mogelijke trend gevonden ten gunste van directe interventie na het stellen van de diagnose en heeft orchidopexie als behandeling de voorkeur.

List of publications

Scrotal ultrasound findings in previously congenital and acquired unilateral undescended testes and their contralateral normally descended testis.
van Brakel J, de Muinck Keizer-Schrama SM, van Casteren NJ, Hazebroek FW, Dohle GR.
Andrology. 2015 Sep;3(5):888-94. doi: 10.1111/andr.12070. Epub 2015 Jul 27.

Acquired undescended testes and fertility potential: is orchiopexy at diagnosis better than awaiting spontaneous descent?
van der Plas EM, **van Brakel J,** Meij-de Vries A, de Muinck Keizer-Schrama SM, Hazebroek FW, Hack WW, Dohle GR.
Andrology. 2015 Jul;3(4):677-84. doi: 10.1111/andr.12044. Epub 2015 Jun 17.

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van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, Hendriks AE, de Jong FH, Hack WW, van der Voort-Doedens LM, Bangma CH, Hazebroek FW, Dohle GR.
J Pediatr Surg. 2014 Apr;49(4):599-605. doi: 10.1016/j.jpedsurg.2013.09.020. Epub 2013 Oct 3.

Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study.
van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, Hendriks AE, de Jong FH, Bangma CH, Hazebroek FW, Dohle GR.
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Point-of-care C-reactive protein testing in febrile children in general practice.
Monteny M, ten Brinke MH, **van Brakel J**, de Rijke YB, Berger MY.
Clin Chem Lab Med. 2006;44(12):1428-32.

Curriculum vitae

Jocelyn van Brakel was born on August 8th, 1982 in Dordrecht, the Netherlands. She attended high school at the Insula college in Dordrecht and graduated in 2001. She started her medical training at the Erasmus University in Rotterdam. After her graduation in January 2008, she worked one year as a junior resident (ANIOS) at the department of Urology at the Erasmus MC. From 2009 up until 2012, Jocelyn worked at the department of Andrology at the Erasmus MC, while simultaneously working on her PhD research in field of Andrology. During this period, she was trained in clinical Andrology according to the European Andrology Association (EAA) guidelines. In 2010, she performed her examination in clinical Andrology in Athens at the congress of the EAA.

In May 2011, she applied for the Urologic traineeship and in January 2012 she started her residency in general surgery for two years at the Albert Schweitzer hospital in Dordrecht under supervision of Dr. P.P. Plaisier. In 2014, she performed the academic part of her urologic traineeship at the Erasmus MC under supervision of Dr P.C.M.S. Verhagen. The last two years of her urological training will be spent at the Amphia hospital in Breda under supervision of Drs. D.K.E. van der Schoot.

PhD Portfolio

Name	Jocelyn van Brakel
PhD Period	2009-2016
Erasmus MC Department	Urology/Andrology
Promotor	Prof. Dr. C.H. Bangma
Supervisors	Prof. Dr. F.W.J. Hazebroek, Dr. S.M.P.F. De Muinck Keizer-Schrama, Dr. G.R. Dohle

PhD training	**Year**	**ECTS**
General courses		
Methodologie van Patient gebonden Onderzoek en voorbereiding van subsidieaanvragen	2009	0.3
BROK ('Basiscursus Regelgeving Klinisch Onderzoek'	2009	0.7
English course, Proficiency B class, centre for British English	2009	1.4
English course, "Discussion & speaking skills"	2009	1.2
Biomedical English Writing and Communication	2010	3
Specific courses (e.g. Research school, Medical Training)		
Microchiriurgische vaso-vasostomie cursus	2008	0.3
Erasmus Winter Programme: Introduction to Clinical Research	2009	0.9
Erasmus Summer Programme: Introduction to Data-analysis	2009	0.9
Erasmus Summer Programme: Regression Analysis	2010	1.9
Seminars and workshops		
Young Urology Masterclass, presentation training	2009	0.3
Young Urology Masterclass, writing research proposal	2010	0.3
Presentations		
Podium sessions		
- Externe refereeravond Urologie, Rotterdam	2010	2
Fertiliteitsscreening van mannen met een voorgeschiedenis van niet-ingedaalde testis		
- 62th Kongresses der deutschen Gesellschaft fur Urologie, Dusseldorf	2010	2
Does age at orchiopexy influence future semen parameters?		
- 6th European Congress of Andrology, Athens	2011	2
Semen analysis in men with a history of congenital or acquired undescended testes		
- Wetenschapsdag Voortplantings centrum, Rotterdam	2011	2
Niet ingedaalde testes: is vroege orchiopexy de sleutel tot een verbeterde fertiliteit?		
- 26th Annual European Association of Urology congress, Vienna	2011	2
Does age at orchiopexy influence future semen parameters?		
- NVU (Dutch Society Urology); Voorjaarsvergadering	2011	3

1. Niet ingedaalde testes: is vroege orchiopexie de sleutel tot verbeterde fertiliteit?
2 Verschillen in operatieve bevindingen bij aangeboren en verworven niet scrotale testes.
- Symposium Kinder chirurgie 2013 2
Evaluatie van fertiliteitparameters bij mannen met congenitale of verworven niet scrotale testis; een lange termijn onderzoek
- Voorjaarsvergadering Nederlandse vereniging van Heelkunde 2014 2
Evaluatie van fertiliteitparameters bij mannen met verworven niet scrotale testes

Poster presentation
- 27th Annual European Association of Urology congress, Paris 2012 2
Fertility in men with acquired undescended testis, where spontaneous testicular descent was awaited until puberty; spontaneous descend versus orchiopexy
- 9th joint meeting European Society for Paediatric Endocrinology Mlian 2013 2
Fertility screening in men with acquired undescended tests: a long-term follow-up study

<u>Academical work</u>
- Journal club monthly 1
- Campell's club monthly 1
- 'promovendi avond' monthly 1
- Interne & externe refereer avond', Erasmus MC, Rotterdam monthly 1

Teaching
Lecturing
Supervising practicals and excursions, Tutoring
Microchirurgische vaso-vasostomie cursus 2009 0.3

Other
European Andrology academy. 2011 10
Two year training and exam clinical andrology