From THE DEPARTMENT OF WOMEN'S AND CHILDREN’S HEALTH
Karolinska Institutet, Stockholm, Sweden

A PROSPECTIVE RANDOMIZED STUDY OF CONGENITAL
CRYPTORCHIDISM

Claude Kollin

Stockholm 2012
ABSTRACT

Background: Cryptorchidism is the most common anomaly in newborn boys. Despite its frequency there are controversies regarding many aspects, including the optimal timing of surgery, natural course of the spontaneously descended testes, frequency of acquired cryptorchidism, risk of infertility and testicular cancer. Based on indirect proof the general consensus is that treatment should be offered before the age of 2 years. However, this has not been proven in prospective randomized trials.

Aims: The objectives are to present the first prospective randomized study on the optimal age for treatment, to identify markers of importance for future spermatogenesis and to investigate the growth and natural course of the spontaneously descended testes.

Patients and methods: A total of 426 boys were included. Morphometric, volumetric and endocrine data were investigated in boys randomized to surgery at 9 months or 3 years of age and compared to boys with spontaneous descent up to 5 years of age. A total of 213 biopsies were analyzed, testicular volume was assessed from ultrasound measurements at birth and 6 months and then yearly up to 5 years of age. At surgery, measurements were made with a ruler. Inhibin B, LH, FSH and testosterone were analyzed at birth, at 2 and 6 months and at 1, 2 and 4 years of age.

Results: Comparisons between early and late treated boys showed significant differences in testicular growth, number of germ and Sertoli cells (p<0.001) at the time of surgery. The testes that descended spontaneously showed impaired growth and were found to be at high risk for later ascent. No conclusive differences in hormone levels were demonstrated.

Conclusions: We have proven that surgical treatment at 9 months of age is more beneficial for spermatogenesis and testicular growth compared to treatment at 3 years of age, testicular volume was also shown to reflect spermatogenesis in early childhood. Hormones taken at infancy could not predict later testicular growth or spermatogenesis. We proved that also spontaneously descended testes have impaired later growth and, furthermore, that many of these testes ascended later on.
ORIGINAL STUDIES

The thesis is based on the following publications (I and II) and original studies (III and IV) and referred to by their Roman numerals.


III. Kollin C, Granholm T, Nordenskjöld A, Ritzén EM. Growth of the undescended testis after spontaneous or surgically induced descent.

IV. Kollin C, Stukenborg JB, Nurmio M, Sudqvist E, Gustafsson T, Nordenskjöld A, Ritzén EM, Toppari J. Boys with undescended testes: Endocrine, volumetric and morphometric studies on testicular function before and after orchidopexy at 9 months or 3 years of age.
# TABLE OF CONTENTS

ABSTRACT ..................................................................................................................... III

ORIGINAL STUDIES ...................................................................................................... IV

TABLE OF CONTENTS .................................................................................................... V

1. Introduction .................................................................................................................. 1
   1.1 Definitions .............................................................................................................. 2
   1.2 Diagnosis .............................................................................................................. 3
   1.3 Congenital cryptorchidism ................................................................................... 5
      1.3.1 Epidemiology .................................................................................................. 5
      1.3.2 Risk factors .................................................................................................... 6
      1.3.3 Spontaneous descent .................................................................................... 6
   1.4 Acquired cryptorchidism ....................................................................................... 6
      1.4.1 Epidemiology .................................................................................................. 6
      1.4.2 Etiology .......................................................................................................... 7
      1.4.3 Treatment ....................................................................................................... 7
      1.4.4 Conclusion ...................................................................................................... 7
   1.5 Retractile testes ...................................................................................................... 8
      1.5.1 Epidemiology .................................................................................................. 8
      1.5.2 Definition ....................................................................................................... 8
      1.5.3 Conclusion ...................................................................................................... 9
   1.6 Normal testicular descent ..................................................................................... 9
      1.6.1 Transabdominal phase, gestational week 6 to 15 ........................................... 10
      1.6.2 Inguinoscrotal phase, gestational week 25 to 35 ........................................... 10
   1.7 Pathogenesis ......................................................................................................... 11
      1.7.1 Genetics ......................................................................................................... 11
      1.7.2 Clinical conditions and syndromes ................................................................ 11
      1.7.3 Androgen disorders ...................................................................................... 12
      1.7.4 Environmental and maternal factors ............................................................. 12
   1.8 Testicular cancer .................................................................................................... 13
      1.8.1 Cryptorchidism and malignancy .................................................................... 13
      1.8.2 Age at treatment and malignancy .................................................................. 14
      1.8.3 Carcinoma in situ .......................................................................................... 14
      1.8.4 Conclusion ...................................................................................................... 15
   1.9 Hormonal treatment ............................................................................................. 15
      1.9.1 Modes of treatment ....................................................................................... 15
      1.9.2 Efficacy .......................................................................................................... 16
      1.9.3 Adverse effects ............................................................................................. 16
      1.9.4 Conclusion ...................................................................................................... 16
   1.10 Surgical treatment ............................................................................................... 17
      1.10.1 Success rates ............................................................................................... 17
      1.10.2 Management of the palpable testis ............................................................... 17
      1.10.3 Management of the non-palpable testis ....................................................... 18
      1.10.4 Recurrences .................................................................................................. 21
8. Conclusions and future perspectives..........................................................................................86
9. Acknowledgements..................................................................................................................89
10. References ..............................................................................................................................92

Original Studies I-IV..................................................................................................................99
1. Introduction

Cryptorchidism is a condition where one or both testes fail to descend into the scrotum and the most common anomaly in newborn boys. Despite its frequency there are still controversies regarding many aspects, including etiology, natural history, mode and timing of treatment as well as the risk of infertility and testicular cancer.

Almost all controversies are the result of a lack of standard definitions and prospective long-term follow-up studies, which makes it very difficult to compare results from the numerous studies done on cryptorchidism.

There is a general consensus that the condition should be treated early in life to reduce the risk of infertility and testicular cancer. However in reality, we do not know whether or not treatment in early infancy reduces the risk of testicular cancer or to what extent fertility is improved, especially when only one testis is affected. Is surgery performed only to make it easier to detect a future testicular cancer and for cosmetic reasons in these cases?

The proper timing of surgery has long been debated. In the seventies the American Academy of Pediatrics recommended that genital surgery should be done at the age of four for psychological reasons. During the last decades, based on indirect proof, there has been a shift towards earlier treatment leading to a general consensus that treatment should be given before the age of two. However, this has yet to be proved in prospective studies where boys are randomized to surgery at different ages and then followed through childhood and puberty to assess the success of the treatment.

The main objective of this thesis report is to present a prospective randomized study on the timing of treatment in congenital cryptorchidism.
1.1 Definitions

Incomplete descent of the testes is the most common congenital anomaly in boys, but the defect can also be acquired during childhood. Most studies on cryptorchidism are retrospective, lack standard definitions and nomenclature for different forms of undescended testes and diagnose. In this thesis report we have classified the boys examined according to their phenotype and used a clear and consistent way of diagnosing the condition.

Cryptorchidism can either be congenital or acquired. At examination the testis can be classified as either uni- or bilateral and palpable or non-palpable in different combinations. A retractile testis is a testis that temporarily resides above the scrotum due to the cremaster muscle activity.

Fig. 1. Shows the possible locations for an undescended testis.

A palpable undescended testis can be found somewhere along the normal path for descent in the inguinal canal, whereas an ectopic testis, on the other hand, has deviated and can often be found at other locations in the inguinal and pubic regions or even in the perineum on rare occasions. An ectopic testis can be diagnosed at clinical examination but is commonly diagnosed during surgery. An iatrogenic
*retained testis* is a testis that was previously located in the scrotum, but has become retained after inguinal surgery such as hernia repair. In cases of a *non-palpable unilateral testis* the testis is either an *intraabdominal testis* or, if the testis is missing, referred to as *monorchia*, or if both testes are missing the condition is called *anorchia*.

![Image](image.png)

Fig. 2. The figure shows a 9-month old boy with unilateral cryptorchidism before and at orchidopexy.

### 1.2 Diagnosis

When determining the position of the testes in clinical practice it is often quite obvious that a testis is non-palpable or in a retained, position and cannot be manipulated into the scrotum.

Sometimes, however, it is difficult to assess whether the testis is in a retained position or not, and the physicians' decision is then often based on his or her
personal experience. The physician has to take into account where the testis is located without manipulation, how far down the testis can be manipulated and finally how long it will stay in its lowest position after the traction has been released. The position during and after traction also depends on other factors such as the experience of the physician, how much tension can be allowed, and how well the boy cooperates.

Consider the following example, which illustrates the difficulties and highlights the need for standard definitions that will work in clinical practice: If a testis is spontaneously located in the groin and can be pulled to the upper or lower part of the scrotum and held there rather comfortably for the boy, but when the traction is released the testis retracts back to its original position. Is this a retained testis or a highly retractile one? Alternatively if the testis would stay in the scrotum for a couple of seconds, would that change the decision? We can easily comprehend that not all physicians will take the same decisions or in other words, some boys will be referred to surgery and some boys will be discharged with no further follow-up.

*The studies presented in this thesis report strictly use the following clinical definition of a palpable undescended testis*

The patient is examined in supine position with legs slightly bent. We determine the position of the testis at one of three levels using the middle of the testis as a reference point for each position: suprascrotal, (SS) upper portion of the scrotum (US) or lower portion of scrotum (LS). If a testicle spontaneously assume a suprascrotal position and cannot be pulled down into the scrotum, we categorize this as an undescended testis. If a testicle in a suprascrotal position can be pulled down into the scrotum, but will not remain there after about 30 seconds of traction (to exhaust the cremasteric reflex), we define this as an undescended testis as well. If the testis remains in upper or lower scrotum position for approximately 10 seconds or longer after the traction had been released, it is defined as a retractile testicle.
1.3 Congenital cryptorchidism

1.3.1 Epidemiology

The prevalence of congenital cryptorchidism at birth is 2 – 9 % with apparent geographical differences in the Nordic countries [1, 2]. Surprisingly in Denmark the prevalence at birth is 9 %, whereas in Finland it is 2 – 3 %. Authors suggest that the difference in prevalence between the countries may be attributed to genetic, endocrine, environmental and lifestyle factors, as will be discussed later. Unilateral cryptorchidism accounts for more than 80 % of all cases and bilateral cryptorchidism approximately for 20 % [3].
1.3.2 Risk factors

Risk factors for cryptorchidism are low birth weight <2500 g), prematurity and small for gestational age and the condition is also associated with a higher incidence of other congenital abnormalities of the external genitalia [4, 5].

1.3.3 Spontaneous descent

Due to spontaneous descent that occurs mainly within the first three months of life the prevalence at the age of one year is 1 – 2 %. Spontaneous descent is more likely to occur in preterm boys and infants with low birth weight. [3-6].

1.4 Acquired cryptorchidism

1.4.1 Epidemiology

The prevalence of cryptorchidism seems to increase with age, thus leading to an increased number of orchidopexies with an accumulated prevalence of 2 – 4 %. In one study the prevalence, without treatment, at ages 6, 9 and 13 years were 1.2 %, 2.2% and 1.2 % respectively [7]. On average orchidopexy is performed at the age of four, in other words, at a much higher age than is currently recommended for treatment. This clearly indicates that descended testes can ascend to a position defined as cryptorchid. Highly retractile testes are suggested to be more prone to ascend

Some researchers define testes that at birth are cryptorchid followed by spontaneous descent and then become cryptorchid again as recurrent cryptorchidism, to be distinguished from true acquired cryptorchidism where the testes are normal at birth. The John Radcliffe Hospital group found that a considerable number of the testes that spontaneously descended were again in a cryptorchid position when the boys were one year old. They came to the
conclusion that this explains why so many orchidopexies were done compared to the incidence of cryptorchidism found at the age of one [8-10].

1.4.2 Etiology

The etiology of acquired or recurrent cryptorchidism is unclear. Some researchers have proposed that processus vaginalis open or as a fibrotic strand, can allow ascensus to occur. Others have proposed that an overactive cremasteric muscle (retractile testes) activity may be the cause, or that a relative cranial migration of the testis may be the result of a discrepancy between the growth of the testicular cord and the linear somatic growth of the patient [11-13].

1.4.3 Treatment

Several authors advocate a wait-and-see approach since a considerable number of ascending testes (57 - 78 %) will descend spontaneously by puberty. However, testes that do not descend require surgery and are often smaller than the contralateral testis. [10, 14-17]. Histopathological studies on acquired forms of cryptorchidism show that they undergo germ cell deterioration, in the same manner as in congenital undescended testes [18, 19].

1.4.4 Conclusion

Testes in acquired cryptorchidism will be exposed to the same adverse conditions in the groin as true cryptorchid testes and consequently some of them will have impaired growth and spermatogenesis. No prognostic factors in childhood have been identified by which we could assess whether a testis will descend by puberty or not. Based on these data it has been recommended to consider surgical treatment upon presentation [20].
1.5 Retractile testes

1.5.1 Epidemiology

In clinical practice retractile testes are often regarded as normal. The parents are often assured that the condition will correct itself and thus their boys are frequently discarded from further follow-ups. Retractility is attributed to the activity of the cremaster muscle and is absent during the first months of life. As in acquired forms of cryptorchidism a major part of these testes (77 %) will descend spontaneously into the scrotum by puberty [21]. Retractile testes are most common in the pre-school period with a peak period around 5 - 8 years of age. The peak period coincides with the period when the prevalence of acquired cryptorchidism is high, thus suggesting that retractile testes are often misdiagnosed as truly undescended testes [22].

Furthermore, histological studies show that retractile testes exhibit the same adverse histological changes as cryptorchid testes and may therefore be a cause of subfertility with abnormal spermiograms. Retractility was found more frequently in infertile men treated for undescended testes in childhood than in the population in general: 30 versus 12 % [23-26]. A recent study of retractile testes in boys has shown that the testicular volume was lower than in boys with scrotal testes [27].

1.5.2 Definition

The definition used in our studies is discussed in the section on diagnosis. Attempts have been made to define a retractile testis in adults. In one study the authors defined a retractile testis as one that at least once a week ascends to a position above the scrotum [24]. However, in our point of view it would be almost impossible to practise this definition on boys since there is no objective way of quantifying the accumulated time in a suprascrotal position.
Consequently there are highly retractile testes that almost never stay in the scrotum during an ill-defined period and there are testes that remain in place most of the time. This results in a clinical dilemma: which boys should we treat and which boys should be followed or discharged from further follow-up? The highly retractile testes that occur in periods with high cremaster muscle activity may very well be what many physicians would regard as cases of acquired cryptorchidism.

1.5.3 Conclusion

So in summary retractile testes, congenital and acquired forms of undescended testes can be difficult to distinguish from each other and is therefore sometimes diagnosed incorrectly. In addition, all forms are at risk for impaired spermatogenesis. Some testes in acquired cryptorchidism will descend by puberty however no prognostic factors in childhood have been identified by which we could assess whether a testis will descend or not.

Numerous studies on these different forms of cryptorchidism are retrospective, which makes it very difficult to assess and compare the results. The lack of standard definitions results in a mix of different forms of undescended testes in patient materials, which emphasizes the need for prospective studies with clear definitions and nomenclature.

1.6 Normal testicular descent

Testicular descent is a complex process that only occurs in mammals. Most data are collected from animal studies and therefore not always easy to translate to conditions in man. However, it is widely accepted that testicular descent occurs in two separate phases: firstly the non-androgen dependent transabdominal phase and secondly the androgen-dependent inguino-scrotal phase, where the second phase is considered more complex than the first [28, 29].
1.6.1 Transabdominal phase, gestational week 6 to 15

When the embryo is six to eight weeks the testes are formed from undifferentiated gonads, close to the kidneys. The testes secrete AMH (anti müllerian-hormone) and testosterone. AMH causes regression of the Mullerian ducts, preventing the boy to develop internal female genitalia (uterus and fallopian tubes). Testosterone causes regression of the cranial suspensory ligament (CSL), a ligament that anchors the testes close to the kidneys. Simultaneously the Leydig cells produce INSL3 (insulin-like hormone peptide) that causes enlargement and swelling of the caudal ligament, the gubernaculum. The masculinisation process also depends on a functioning receptor for INSL3, LGR8 [30]. These events lead to attachment of the testes close to the internal ring, preventing the testes to ascend to a position high in the abdomen as ovaries do with the enlargement of the embryo [31].

1.6.2 Inguinoscrotal phase, gestational week 25 to 35

During the inguinoscrotal phase the gubernaculum elongates and migrates with the testis from the inguinal region through the inguinal canal into the scrotum. Androgens regulate the migration by acting through the calcitonin-gene-related peptide (CGRP), which is a neurotransmitter that acts chemotactically on the gubernaculum. CGRP is released by the genitofemoral nerve (GFN), and considered to be a key regulator in the second phase of testicular descent. Animal studies have shown that transection of the genitofemoral nerve results in cryptorchidism [29].

The migration of the testis through the inguinal canal is also considered to depend on abdominal pressure. In vitro studies show that CGRP may obliterate the processus vaginalis after completed descent [28]. If this is of importance in the pathogenesis of undescended testis is unclear, but it is interesting to note that boys operated on for undescended testes often have a patent processus vaginalis. The second phase may also be affected by other factors such as Hox 10 [32, 33].
1.7 Pathogenesis

The pathogenesis for cryptorchidism is considered to be multifactorial. Many etiologies have been suggested for congenital cryptorchidism but it occurs mostly as an isolated disorder with no obvious single cause but can be associated with genetic, endocrine and somatic disorders. Several environmental and maternal factors have also been proposed to play a role.

1.7.1 Genetics

In animal studies on knockout XY mice for the INSL-3 gene are found to be cryptorchid with impaired spermatogenesis [34, 35]. In man mutations of the INSL3-gene and its receptor LGR8-gene rarely cause cryptorchidism - only a few percent of all cases. Epidemiological studies on familial cryptorchidism show that brothers of cryptorchid boys have a 6 % risk and fathers about 4 % compared to the population. In bilateral cases even a higher risk of recurrence is revealed [36, 37]. One explanation for the low frequency of mutations in INSL-3 gene and its receptor may be attributed to the fact that the first phase of testicular descent seldom fails or that many other genes are involved, which are not yet identified.

1.7.2 Clinical conditions and syndromes

Persistent müllerian duct syndrome is a rare condition where a boy who has bilateral cryptorchidism also has internal female genitalia. This syndrome is caused by a mutation in the AMH gene or its receptor for anti-müllerian hormone (AMH) that prevents the regression of the müllerian ducts resulting in a uterus and fallopian tubes in an otherwise normal boy.

Abdominal pressure is considered to be important in the second phase of descent, interestingly infants with low abdominal pressure in conditions like gastrochisis have a higher incidence of undescended testes [38]. Syndromes with muscular hypotonia such as Down, prune belly and Prader Villi also have a higher incidence
of cryptorchidism. The same can be said about patients with spina bifida in the caudal segment, suggesting that the genitofemoral nerve play a role, not only in animal models but also in man. Cryptorchidism is also associated with a higher incidence of hypospadias and ambiguous genitalia [39].

1.7.3 Androgen disorders

The second phase of normal testicular descent is androgen dependent and therefore various androgen deficiencies have a higher incidence of congenital cryptorchidism. The mechanisms include inadequate production or action of androgens as in hypogonadotropic hypogonadism and mutations or a deficient action on the androgen receptor.

1.7.4 Environmental and maternal factors

Estrogens, pesticides and phthalates have all been proposed to play a role in cryptorchidism. For instance, pesticides in breast milk are associated with congenital cryptorchidism. Some researchers suggest that estrogens in the environment may cause a disrupted ratio between androgens and estrogens leading to an inadequate maturation of Sertoli and Leydig cells. However, these studies are not conclusive and show contradictive results [32, 40-42]. Maternal factors such as smoking and alcohol consumption during pregnancy have been studied and considered to cause bilateral cryptorchidism, but the mechanisms for these associations are not known. It has been speculated that the accumulative effects of many different exposures may have adverse consequences especially during fetal life and childhood [43-45].
1.8 Testicular cancer

1.8.1 Cryptorchidism and malignancy

Decades ago it was assumed that the relative risk of testicular cancer was 40 - 50 times higher in men treated for cryptorchidism than in the general male population. Today, based on cohort studies, the relative risk is considered to be 4-8 fold times higher. In bilateral undescended testes the relative risk to develop malignancy in each testis is slightly higher than in unilateral cryptorchidism [46-49].

Another issue discussed is whether the contralateral testis in unilateral cryptorchidism is at higher risk for developing future malignancy. The results obtained are somewhat contradictory, but generally there seems to be a slightly increased risk [46, 50, 51].

In Denmark the prevalence of cryptorchidism at birth is 9 %, higher than in other Nordic countries, interestingly men in Denmark also have a higher incidence of testicular cancer and impaired semen quality. The cause of the geographical difference is unclear but may be attributed to genetic, maternal, lifestyle or environmental factors [2, 41, 45, 52].

Men with untreated cryptorchidism is at higher risk for developing seminomas, whereas treated men are more likely to develop nonseminomatous tumors [53].

Removal of the testis is recommended in adult men with unilateral cryptorchidism since studies show highly impaired spermatogenesis at this age and also a significant malignancy potential in the removed testes [54].
1.8.2 Age at treatment and malignancy

There are no published studies that conclude that orchidopexy in early infancy reduces the risk of malignancy. Testicular cancer usually occurs in men over 30 years, so consequently the current cohorts on malignancy are based on how cryptorchidism was treated decades ago - that is at a much higher age than recommended today.

Some studies have not been able to correlate age at surgery with testicular cancer [55]. On the other hand, a Swedish study shows that the relative risk of developing testicular cancer is 2.23 times higher if treatment is given before the age of thirteen, compared to later treatment where the relative risk is 5.4 [56]. These figures are also confirmed in other studies [57].

1.8.3 Carcinoma in situ

Carcinoma in situ or CIS is a precursor of testicular malignancies. Authors have hypothesized that CIS originates during fetal development. It results in a defect transformation to spermatogonia leading to pluripotent stem cells that are at higher risk for malignant transformation [58].

The prevalence of CIS in men with a history of cryptorchidism is 2 - 4 % and less than 1 % in the general population, which is similar to the lifetime risk of developing testicular cancer with preceding carcinoma in situ [46, 47, 50, 59]. In men with testicular cancer a biopsy on the contralateral testis showed that the prevalence of CIS was 5 % and the risk of progress to invasive malignancy was 50 % in 5 years [60-62].

In a cohort study Swerdlow et al. conclude that the biopsy itself is a risk factor for malignancy in the biopsied testis. However, these results are widely disputed and no relation has been proven in other cohorts [50, 61]. Microcalcifications and its
relevance for future malignancy have been discussed and there is no agreement how these should be followed clinically and recommendations vary [63, 64]. Some studies show a correlation between testicular microcalcifications (TM) and carcinoma in situ [65, 66], but other researchers conclude that TM alone is not premalignant in itself [67].

1.8.4 Conclusion

Cryptorchid boys are at higher risk for developing carcinoma in situ and testicular cancer as adults. Published data reflects the time when almost no surgical treatment was undertaken before the age of two. Orchidopexy before puberty clearly reduces the above-mentioned risks, but it remains to be proven that orchidopexy before the age of one reduces the risk even further.

1.9 Hormonal treatment

During the last decade hormonal treatment for cryptorchidism has lost popularity in Europe due to a low efficacy rate and a highly potential risk for adverse effect on the testis itself. Therefore this mode of treatment will only be discussed briefly in this report.

1.9.1 Modes of treatment

Treatment options include the use of human chorionic gonadotrophin (hCG) and gonadotrophin-releasing hormone (GnRH). These hormones have been used separately or in combination, and also prior to or after surgical treatment. Both hCG and GnRH (through LH release) stimulate the Leydig cells to produce testosterone.

Some researchers suggest that postoperative hormonal treatment has beneficial effects on sperm counts in adulthood [68, 69]. In one study boys were randomized
either to surgery only or to GnRH treatment prior to orchidopexy. The results show that treatment prior to surgery improves the number of dark spermatogonia per tubule. [70]. These findings were confirmed by Hadziselimovic et al. who showed in a study that hormonal treatment prior to surgery induces the maturation process to dark spermatogonia. Also, the results could be directly correlated to fertility potential [71]. So far, however, the latter have not been confirmed in other studies.

### 1.9.2 Efficacy

Four different modes of hormonal treatment were evaluated in a study with an overall success rate of about 20 %. The recurrence rate six months after treatment was 23 %, which confirms the results from several meta-analyses, showing even lower efficacy rates if retractile testes were excluded. The results were the same for all the different modes of treatment [72-75].

### 1.9.3 Adverse effects

HCG treatment has proved to have adverse effects on the testis itself, causing germ cell apoptosis and lesser testicular volume in adulthood [76-79]. In addition, multiple intramuscular injections treatment with hCG has clinical side effects such as acute inflammatory reaction of the testes, enlargement of the genitalia, pubic hair growth and possible aggressive behaviour of the child. Treatment with GnRH has not presented any clinical side effects [20].

### 1.9.4 Conclusion

Due to the low efficacy rate and the adverse effects after hormonal treatment on the testis, the Nordic consensus report recommends surgery as the primary choice of therapy for undescended testes. Hormonal therapy may be considered in cases where surgery is not an option. At present, no conclusive evidence supports
hormonal treatment before or after surgery. More studies on this subject are required [20].

1.10 Surgical treatment

1.10.1 Success rates

The short-time definition of success after orchidopexy is a scrotal position of the testis without atrophy or recurrence, whereas the long-term definition of success would be preserved functionality. A successful orchidopexy is not a guarantee for optimal functionality, and in some cases a testis is fixated in a position above the scrotum just to preserve hormonal functionality and assure palpability.

The success rate varies depending on the location of the testes, experience of the surgeon, age of the patient at time of surgery and other factors as well. A meta-analysis by Docimo et al. of 64 studies shows that the success rate for palpable inguinal testes is approximately 90 % and for abdominal testes 74 %. Success by procedure was approximately 90 % for an inguinal approach, and 70 - 80 % for the different techniques used for the intraabdominal testes. During the last decade success rates have improved with new surgical approaches and more experience of different techniques [53, 80, 81].

Management of the palpable and the non-palpable testes and a relatively short description of the different surgical options and techniques are presented in this report. For more details see review by Thorup et al. [82].

1.10.2 Management of the palpable testis

Surgical management of unilateral or bilateral palpable testes aims to create a Darto’s pouch in the scrotum, which can be achieved by a single scrotal technique or a two-incision technique. The single incision technique or transscrotal approach
is often used for more distal testes as described by Bianchi, and is preferable for surgeons familiar with the technique [83]. The two-incision technique involves an incision in the groin and one in the scrotum to create the pouch. In this prospective study the latter has been the method of choice for all palpable testes. The technique is described in more detail in the section on methods.

1.10.3 Management of the non-palpable testis

According to present recommendations orchidopexy should be performed between six and twelve months of age by an experienced pediatric surgeon or urologist and by a pediatric anesthesiologist. Initial diagnostic laparoscopy has a high accuracy rate. It is widely used among surgeons as the initial step in the management of the non-palpable testis and provides information regarding the next surgical step. Imaging techniques such as ultrasonography, computed tomography (CT) or magnetic resonance imaging (MRI) are not recommended routinely since they are less sensitive than an experienced physician and not always easily accessible in clinical practice [84]. For young boys the use of MRI and CT is not justified since these methods generally require anesthesia. However, in some situations ultrasonography may be of diagnostic value, for example when a boy is difficult to palpate due to obesity or a lack of cooperation.

Findings at laparoscopy and surgical management

In boys with non-palpable testes laparoscopy revealed an intraabdominal testis in 36% of the cases, blind ending vessels in 44% and an inguinal testis or remnant in 20% of the cases. The next surgical step is based on the intraoperative findings at laparoscopy [85].

Blind ending vas deferens and vessels: In unilateral cases no further exploration or evaluation is needed whereas in bilateral cases the boy should be referred to a pediatric endocrinology unit for evaluation and future hormonal substitution.
Vas deferens and vessels exiting a closed or open internal ring: In these cases an inguinal exploration should follow after laparoscopy. If the internal ring is closed there is almost certainly a remnant or nubbin in the inguinal canal. This remnant should be extirpated since it has been shown that viable germ cell tissue exists in a few percent of all remnants with a risk of progressing to a future malignant transformation. However, this has not been proven in follow-up studies. An open internal ring is a clear indication that there is a viable testis in the canal [86, 87].

Fig. 4. On the left the vas deferens and spermatic vessels are entering a closed internal ring in a boy with a viable testis in the inguinal canal. On the right the vessels are ending blindly in a monorchid boy

Unilateral intraabdominal testis: The testis can be located very close to the internal ring it is sometimes referred to as a peeping testis or a low abdominal testis, or if it is located higher in the abdomen a high intraabdominal testis.

A testis close to the internal ring or even a higher located testis can often be brought down to scrotum by a high inguinal incision followed by an extensive retroperitoneal dissection creating sufficient length for a scrotal orchidopexy [88].
If the surgeon during laparoscopy finds that sufficient length for a scrotal orchidopexy cannot be achieved a Fowler-Stephens procedure is sometimes chosen, meaning the spermatic vessels need to be divided. The blood circulation to the testis will then depend on collateral circulation from the vas deferens, gubernaculum and cremasteric muscle fibres. A Fowler-Stephens procedure can be done either openly or laparoscopically in a single-stage or a two-stage procedure. The two-stage procedure means that firstly the testis is left in an intraabdominal position without tension in order to optimize the development of collateral blood circulation. The second stage or the actual orchidopexy should be done at least six months after the ligation of the vessels. The single-stage procedure means that vessels are divided and an orchidopexy is performed in the same surgical procedure.

It has been shown by Thorup et al. that germ cells may survive the ligation of the vessels, whereas the number of spermatogonia decreases, which is also confirmed in an animal model. Therefore testicular atrophy and/or impaired spermatogenesis are potential complications of this technique [89, 90].

Consequently, the challenge for the surgeon during laparoscopy is to assess whether or not it is possible to bring the testis down to the scrotum without dividing the vessels, thereby reducing the risk of impaired spermatogenesis. The decision must be based on the surgeon's personal experience since no objective methods have been described and randomized prospective studies are lacking.

If inguinal exploration is chosen after laparoscopy and sufficient length cannot be achieved and a subsequent Fowler-Stephens orchidopexy is performed, this would in our opinion not be optimal since the dissection in the groin itself most likely will compromise the collateral circulation to develop and therefore the risk for atrophy increases further.
In case of a solitary intraabdominal testis in monorchid boys most surgeons would probably not perform a Fowler-Stephens orchidopexy due to the risk of atrophy and subsequent androgen substitution throughout life. The general consensus is that the testis should be placed in a palpable position in the lowest possible location without tension.

Orchidectomy can be considered in cases of uncorrected unilateral cryptorchidism in men with a normally descended testis and a contralateral high abdominal testis. The reason is a severely compromised fertility and higher risk of testicular cancer (as discussed later in the section on testicular cancer) in the retained testis.

*Bilateral intraabdominal testes:* The management of bilateral high intraabdominal testes probably varies among surgeons depending on personal experience and preference. Most surgeons would correct one side at a time with at least 6 months wait in between surgeries. The first side should be evaluated in regard to possible atrophy and/or recurrence before the contralateral side is operated on and the appropriate method is chosen.

Other surgical techniques used in the management of the intraabdominal testis have been described such as laparoscopically or robot assisted orchidopexy and autotransplantation of the testis. Autotransplantation, or microsurgery, is a method where the spermatic vessels are anastomosed with vessels located in the groin. In early orchidopexy the use of this technique is limited due to a small diameter of the vessels. Also, the technique requires a skilled surgeon.

### 1.10.4 Recurrences

Recurrences are well known and occur more often after surgery of intraabdominal testes than more distal testes. The experience of the surgeon is also of major importance. In our opinion recurrences often occur when using the inguinal approach as a result of an inadequate retroperitoneal dissection with the
consequence of persistent tension and subsequent retraction to a suprascrotal position. The re-operation carries a higher risk of damaging the vas deferens and the vessels due to scarring tissue and deranged anatomy [91].

1.10.5 Conclusion

In conclusion the success of surgery is defined as a scrotal position with no atrophy and recurrence thus optimizing the potential for spermatogenesis and fertility potential of the testis. The management of palpable testes is usually fairly straightforward. In cases with intraabdominal testes, however, the method chosen for orchidopexy varies more and is often based on the surgeon’s personal experience and preference for the different techniques. More prospective randomized trials are required to evaluate the success rates of the different methods.

1.11 Fertility

1.11.1 Background

The success of surgical treatment is defined as testes in a scrotal position with no atrophy, aiming at improved spermatogenesis. However, the final proof of successful surgery would be normal sperm production in adulthood and achieved paternity. Important parameters to consider when discussing fertility potential are the age at treatment, hormonal parameters, testicular size and semen quality. There are numerous retrospective reports on men with a history of cryptorchidism investigating these parameters.

1.11.2 Age at treatment and fertility

Numerous studies on adults with a history of cryptorchidism have been performed [92-96]. These show that the paternity rate among men treated for bilateral undescended testes is significantly lower compared to men treated for unilateral
cryptorchidism and controls. Some have also demonstrated lower sperm counts, lower inhibin B, and higher FSH and LH levels in men with bilateral cryptorchidism.

Studies on men with a history of unilateral cryptorchidism revealed that the age at treatment, testicular size or location at the time of orchidopexy did not significantly correlate with paternity rate, mean time to conception, semen quality or hormone levels in adulthood compared to men in the general population. However low values of inhibin B were shown to correlate with men having compromised fertility in this group with unilateral cryptorchidism. Investigators have concluded that indicators of high risk for infertility are low levels of inhibin B together with high levels of FSH and decreased sperm counts.

Other studies show a correlation between testicular volume and sperm concentration but not with testicular position and age at surgery [97]. On the other hand, some studies on unilateral cryptorchidism show that treatment before eight years of age lead to higher inhibin B levels, testicular volume and lower FSH values compared to treatment given after this age [98, 99].

An extensive retrospective biopsy study by Cortes et al. on men who were younger than twelve years of age at surgery showed that a lack of germ cells appeared from 18 months of age at surgery. A normal sperm count was observed in 19 % of the men with a history of bilateral cryptorchidism and for unilateral cases the figure was 83 %. There was no correlation between sperm count and the number of spermatogonia in the unilateral group, whereas it could be seen in the bilateral group. If there were no germ cells at orchidopexy it was associated with 80 - 100 % risk of infertility for the bilateral group and 33 % for the unilateral group [100]. This study indicates that surgery should be performed early in life before spermatogonia disappear, and that there is no correlation between semen quality
in men with a history of unilateral cryptorchidism whereas in bilateral cases the correlation is significant. The paternity rate was not investigated.

Another study on men with previously unilateral undescended testes shows that treatment around the age of two results in higher inhibin B and lower FSH levels compared to later treatment, whereas a corresponding difference in semen quality could not be proven [101].

In a unique and interesting study thirteen men treated before one year of age were compared with sixteen men who were operated on between ages one and two. The results showed a significantly higher sperm density and motility in the group who were treated before the age of one. On the other hand, there were no differences between uni- or bilateral cases, testicular volume, and number of spermatogonia or position of the testes at time of orchidopexy. [102]. This seems to be the only retrospective study conducted on men treated at an age in accordance with present recommendations, but it has some limitations: relatively few subjects were included in the study and the reasons to perform orchidopexies before one year of age are not clearly stated.

Another study showed that men with a history of bilateral undescended testes who had had an operation between ten months and four years of age had a normal sperm count in 76 % of the cases compared to 26 % if the orchidopexy had been done between four and ten years of age. In unilateral cases the figures were more moderate: 75 % compared to 71 % respectively [103]. Other investigators as well confirm that surgery before the age of four in bilateral cases lead to better sperm counts than if treatment is given later [104].
Gouveia et al. concluded that subfertile previously cryptorchid men had lower inhibin B values, higher FSH and lower testicular volumes than subfertile men without a history of cryptorchidism and controls. In addition, significantly higher concentrations of inhibin B and sperm were observed in men who had undergone orchidopexy before the age of four compared to those who had received treatment later [105].

Studies show that men with an absent testis (monorchidism) for whatever reason do not have compromised fertility compared to the male population in general [106, 107].

1.11.3 Conclusion

We have not found any prospective randomized studies on the influence of age at treatment with follow-up on fertility parameters into adulthood. However, there are many retrospective reports with contradictory results as regards the parameters of importance to fertility outcome. One of the limitations of these reports is the lack of clear definitions and nomenclature, resulting in a mix of phenotypes, which may have influenced the results. Decades ago, treatment was generally given at a higher age than is currently recommended, which makes it very difficult to assess, on the basis of published studies, whether surgery in infancy improves spermatogenesis. Some studies conducted on fairly young boys indicate that early treatment is beneficial but fail to prove a positive effect on fertility outcome.

There is no doubt that bilateral cryptorchidism should be treated in childhood and data suggest that early treatment is beneficial for future fertility and paternity. The question is how early should surgery be done to achieve optimal fertility potential in boys with bilateral cryptorchidism? Unilateral cryptorchidism accounts for more than 80 % of all cases and early orchidopexy may be of importance for semen
quality although it is not yet determined if this affects the paternity rate. In summary, data are inconclusive on the effect on paternity in unilateral cryptorchidism thus highlighting the need for prospective long-term studies into adulthood.

1.12 Markers of testicular function

1.12.1 Testicular histology

Most centres advocate early treatment of cryptorchidism based on studies suggesting that early treatment is beneficial compared to later treatment. To finally prove that an orchidopexy has been successful, however, normal sperm production and achieved paternity in adulthood need to be verified in prospective, long-term follow-up studies, which we currently lack.

Consequently, an important issue is how to monitor the testicular function after treatment and identify markers of importance for future fertility and paternity. Testicular histology, hormonal markers and testicular volume are markers that can be monitored during childhood, both before and after treatment.

Normal germ cell development

The testis can be divided into tubular and interstitial compartments. The tubular compartment contains the seminiferous tubules where spermatogenesis takes place and consists of Sertoli and germ cells in different stages. The interstitial compartment contains Leydig cells where testosterone is produced.
Germ cell development is a multistage process starting in the fetus and being completed in puberty. During the fetal period gonocytes are formed to fetal spermatogonia. At three months postnatally the fetal spermatogonia start to transform into adult dark spermatogonia (Ad) and are normally completed between six and twelve months of age. The maturation to Ad spermatogonia a few months postnatally coincides with a period of rise in gonadotrophins and testosterone referred to as minipuberty. The surge in testosterone causes Leydig cell proliferation. Early stimulation of gonadotrophins on the testis is important for the Sertoli, germ, and Leydig cell function. The first months of life are associated with an increase in the total number of Sertoli and Leydig cells together with high serum levels [108]. At three years of age the spermatogonia transform to primary
spermatocytes. From this age the testis remains quiescent until puberty when the final steps of spermatogenesis with the transformation to spermatids will occur.

The number of Leydig cells peak at the time of minipuberty, thereafter the number of active Leydig cells decreases to a minimum at the age of two years and then increases again at puberty [109-111]. In a post-mortem study on boys with no history of cryptorchidism the number of Sertoli cells per tubular cross section has been shown to reach a maximum during the first year of life and then decrease in number up to prepubertal ages [23, 75].

Post-mortem studies on boys with no history of cryptorchidism show that the number of spermatogonia decreases from six months of age and increases again from about three years up to puberty [23, 109, 112]. One of these studies also shows that the reduction of germ cells in boys aged 0 - 1 year compared to 3 - 4 year old boys was approximately 5 % for spermatogonia and approximately 15 % for Sertoli cells [23]. The age group examined in this study is fully comparable to the randomization ages of cryptorchid boys in our own series and therefore provides an excellent reference material.

_Germ cell development in cryptorchid testes_

As a result of failure in the maturation process to Ad spermatogonia and to spermatocytes, the germ cell development is abnormal in cryptorchid testes. Hadziselimovic et al. have shown that the defect transformation will lead to a disproportionate decrease of Ad spermatogonia with a substantial long-term risk of infertility. Interestingly, this reduction is more severe than the reduction of the total number of germ cells. In addition there is also a failure in the maturation process that occurs at about three years of age when spermatogonia transform to spermatocytes.
Furthermore, the numbers of Leydig cells are reduced, maybe reflecting a lack of gonadotrophin stimulation. The number of Sertoli cells has also been shown to decrease, which leads to a diminished volume and diameter of seminiferous tubule. Epididymal abnormalities have been observed possibly due to the effect of a reduced number of Leydig cells and a hypothesized reduced paracrine testosterone secretion on the testis and epididymis [71, 113].

It has been suggested that boys with cryptorchidism can be divided in two subgroups: those with Leydig cells that respond with an increase in testosterone levels after hCG stimulation and those with impaired Leydig cells responding with an insufficient increase in testosterone plasma levels. The latter group with the suboptimal Leydig cell capacity will also have a lower number of Ad spermatogonia than the hCG responders [114]. If this is an indication that preoperative hormonal treatment is beneficial for spermatogenesis and whether hormonal treatment will have a long lasting effect remains to be proven.

In untreated cryptorchid boys a further degeneration of the testis occurs and is considered to be caused by an elevated temperature in the groin, compared to a 2-4°C lower temperature in the scrotum [115]. One hypothesis is that heat induces a defective transformation to spermatogonia. This has been confirmed in animal studies where elevated temperatures proved to have adverse effects on the testis [116-118].

1.12.2 Endocrine markers

Inhibin B, gonadotrophins and testosterone during childhood

Inhibin B is a direct marker of the Sertoli cell function, thus reflecting testicular function. Serum inhibin B, gonadotrophins and testosterone increase during the first months of life with a peak at two to three months of age (minipuberty) and coincide with the maturation of germ cells. The first months of life are associated
with an increased expression of Sertoli and Leydig cells together with high serum levels [108].

The secretion of inhibin B is stimulated by FSH, and suppresses FSH release from the pituitary in a negative feedback loop. Thereafter gonadotrophins and testosterone remain low until the onset of puberty, whereas inhibin B remains higher and stays well measurable into adulthood.

During the first months of life inhibin B correlates positively with gonadotrophins and testosterone but at puberty the correlation with testosterone and LH ceases and a clearly negative correlation with FSH develops and persists into adulthood. Thus the correlation is switched by the onset of puberty. Other investigators have not been able to show a correlation between inhibin B and FSH in childhood [108, 119-122].

Hormones and cryptorchidism

Studies have been conducted on boys with cryptorchidism with contradictory results. One investigation showed that there were no significant differences in levels of inhibin B, gonadotrophins and testosterone compared with normal prepubertal boys, also there was no difference between uni- and bilateral cryptorchidism [123]. However, another report showed that boys with unilaterally undescended testes under the age of 4 years had lower basal inhibin B and higher FSH levels than controls. Inhibin B further correlated to the number of spermatogonia in the undescended testes after preoperative hormonal treatment [124].

Another study by Cortes et al. on 25 boys with bilateral cryptorchidism, who had undergone an operation at an average age of 2.5 years, revealed that a reduced number of spermatogonia could be correlated with low levels of inhibin B at the time of surgery. In bilateral cryptorchid boys inhibin B levels correlated negatively
with FSH. The researchers suggested that boys with low inhibin B and FSH levels together with a poor germ cell count should be evaluated in regard to hypogonadotrophic hypogonadism [125].

### 1.12.3 Testicular volume

The increase in testicular volume from approximately two ml before puberty to more than fifteen ml after puberty is mainly attributed to the increase of germ cells and Sertoli cell volume leading to an increased tubule diameter.

Several methods for measuring testicular volume in adolescents have been described and proven useful [126-129]. Testicular volume has always been considered to reflect testicular function. Incomplete spermatogenesis may therefore result in reduced volume and compromised spermatogenesis in adults [24, 97, 130, 131].

In a retrospective study 723 boys with uni- and bilateral undescended testes were operated on before the age of nine but testicular volume could not predict the germ cell count in cryptorchid boys [132]. As shown in a number of studies the testicular volume is often reduced in men with compromised fertility, but not always correlating with spermograms and paternity as we discuss in the section on fertility.

Ultrasound has shown to be a reliable and reproducible method to determine testicular volume, also during the first year of life [133, 134]. In one study on adults, ruler measurements were compared to ultrasound showing that the results of both methods correlated significantly [128].
1.12.4  Semen quality

Semen quality is an important marker for assessing testicular function in men with compromised fertility, but it will only be discussed briefly in this report. Basic semen parameters include motility, vitality, number and concentration and morphology. Testicular function or spermatogenesis is not the only determinant of sperm concentration in the ejaculate. Factors like the duration of sexual abstinence, fever, stress, medical conditions also play a role, and consequently there is a large intra-individual variation [135, 136]. There are numerous retrospective studies on men with previous cryptorchidism with contradictive results concerning semen quality and paternity as we discuss in the section on fertility.

1.12.5  Conclusion

From our point of view repeated biopsies after an orchidopexy to evaluate the actual treatment is not an option, but biopsies taken at the orchidopexy provide valuable data and can be correlated with testicular volume and hormone levels at the time of surgery.

Serum levels of inhibin B and testosterone reflect the output of both testes and are therefore difficult to interpret in unilateral cryptorchidism, which accounts for the majority of all cases. In bilateral cases, however, these figures could prove to be important.

Serum inhibin B is considered to be a marker of Sertoli cell function, but studies on cryptorchid boys show contradictive results. Consequently the predictive value of prepubertal inhibin B for the quality of spermatogenesis in adulthood remains to be established.
In our opinion, measurements of testicular volume seem to be an acceptable surrogate for repeated biopsies since studies show they correlate with testicular function, though not always with spermiograms and paternity. Consequently, it seems reasonable to follow boys before and after treatment and into puberty with longitudinal ultrasound measurements.

### 1.13 Age at treatment

As discussed previously the optimal time for surgery for cryptorchidism has not yet been established, however most agree that treatment should be offered early in life.

The general consensus on treatment before age one is based on mounting indirect proof even though there are no prospective studies where boys actually have been randomized to surgery at different ages and then been followed with markers of testicular function throughout childhood and puberty. Most studies are conducted on children that are older than one year of age, a period when the testis is quite dormant compared to the first months of life.

A single study has actually conducted a follow-up biopsy after initial biopsy taken at time of surgery. A total of 670 biopsies were taken including biopsies on the scrotal testes. The majority of the treated boys were older than 3 years of age. Histological evaluation revealed that at time of orchidopexy the germ cell population was affected in nearly all undescended testes and interestingly also in 30 percent of the normal scrotal normal testes. No difference was noted between uni- or bilateral cryptorchidism except in bilateral intraabdominal cases where the germ cell loss was severe. A repeated biopsy was performed months or years after the initial biopsy in 18 unilateral and 24 bilateral cases. Interestingly after histological evaluation no significant difference in the number of spermatogonia was observed, suggesting that catch-up spermatogenesis did not occur. The only
difference was the size of the tubular diameter. The authors concluded based on these results that no recommendation on age of treatment could be given [137].

One limitation of this study is that the biopsies were taken at a higher age than recommendations today for orchidopexy meaning that the important maturation step to spermatogonia had already taken place in these biopsied boys, in other words the “damage was already done”.

The fact that the contralateral scrotal testes were affected in 30 percent of the cases is difficult to explain and also these results have not been reproduced in other studies. The study is retrospective and thus carries limitations as such, for example boys with acquired undescended testes and boys with retractile testes might very well be included in the patient material hence influencing the results.

However other studies have suggested that early treatment is beneficiary compared to late treatment.

In an excellent retrospective study by Tasian et al 274 biopsies were reviewed and correlated to age at surgery. The mean age for unilateral and bilateral orchidopexy was between three and four years of age. Surprisingly the authors reported a significant 2 percent risk per month of germ cell loss and 1 percent risk per month of Leydig cell loss for each month a testis remained in a retained position. They furthermore concluded that there is a 50 percent risk for more severe germ and Leydig cell los in cases of non-palpable testes. They were unable to do correlation studies on semen, hormonal or paternity parameters [138].

One recent study investigated biopsies taken from 29 intraabdominal testes. Result showed decreased mean diameter of seminiferous tubules and germ cell depletion increasing with age at orchidopexy. Treatment after 3 years of age predicted severe germ cell depletion in over 90 percent of the cases [139].
An extensive retrospective study by Cortes et al showed that lack of germ cells appeared from 18 months of age at surgery, thus indicating that surgery should be performed before this age. Findings in the study could be correlated to semen quality in both unilateral and bilateral cryptorchidism [100].

1.13.1 Conclusion

Even though results show contradictory results, most studies indicate that the earlier the surgery is performed the less is the germ and Leydig cell depletion, the intraabdominal testes being the worst off.
2. Aims

2.1 Study I and II

To investigate whether surgical treatment at nine months compared to treatment at three years of age of boys with palpable congenital unilateral cryptorchidism is followed by an improved testicular growth up to the age of four years.

2.2 Study III

To examine the natural course of spontaneously descended testes and investigate whether the testicular growth from birth to five years of age are different to scrotal testes and to testes surgically treated at nine months and three years.

2.3 Study IV

To investigate in morphometric and volumetric studies whether surgery at 9 months would be more beneficial than at 3 years and furthermore identify possible endocrine markers for future testicular growth and spermatogenesis in infancy.
3. Study design

The randomized study began in 1998 at Astrid Lindgren’s Children’s Hospital at the Karolinska University Hospital. All the departments of obstetrics in Stockholm were informed about the study and were invited to refer all newborn boys with a suspicion of congenital cryptorchidism to the Department of Pediatric Surgery.

Boys with recognized syndromes, other birth defects, or other pathological conditions afflicting the external genitalia, prematurity (<37 weeks of gestation) or prior groin surgery were excluded from the study.

After confirmation of congenital cryptorchidism and consent from the parents the boy was enrolled in the study. The major part of the boys were included at age 0-3 weeks, however some boys were not included until age 6 months due to late referral or missed appointments. According to their neonatal record these late referred boys were cryptorchid from birth.

The boys were followed with clinical examinations, blood samplings for hormone evaluation and ultrasound examinations at 0-3 ages weeks and at ages 2 and 6 months, 1 one year and yearly up to 5 years of age (all time points +/- 2 weeks) and thereafter at 11 years of age. The data in this thesis report include follow-up until 5 years of age.
Table 1. Study design from birth up through puberty. Data gathered up to 5 years of age is presented in this thesis report (study I to IV).

### Study design

<table>
<thead>
<tr>
<th>+=-2 weeks</th>
<th>Clinical exam.</th>
<th>Ultrasound</th>
<th>FSH, LH, Testosteron, Inhibin B</th>
<th>Randomis. 9 mo/3 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-3 weeks</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>12 months</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Yearly to 5 yrs, 11 yrs and after puberty</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

### 3.1 Ethical permissions

The Ethics Committee of the Karolinska Institute approved the studies and written consent was obtained from the participating families.

### 3.2 Phenotypes and groups

*Enrolment ages 0-3 weeks and 6 months*

The included boys were sub-grouped into palpable or non-palpable uni- or bilateral cryptorchidism.

Some boys could not be classified due to an inhomogeneous phenotype; for example one palpable testis and a non-palpable contralateral testis, these were included in a “mixed” group.
Follow-up at 2 months

Boys that showed spontaneous descent at this age were included and were seen again at age 1 year.

Follow-up at 6 months (randomization age)

At age 6 months the boys that still were cryptorchid were randomized to surgery at either 9 months or 3 years of age. Boys with ascent after initial spontaneous descent (recurrent cryptorchidism) were included upon presentation.

Some boys changed group and were added to the mixed group due to an acquired inhomogeneous phenotype; for example a boy with spontaneous descent one side and cryptorchid on the contralateral side. Consequently the mixed group changed in the number of included boys during the follow-up period until the boys were operated on.

Surgery at 9 months or 3 years of age

At surgery the non-palpable group was separated into uni- or bilateral intraabdominal or monorchid groups. Boys with recurrent cryptorchidism were operated on upon presentation.

Change of study design in 1998

The Nordic consensus report published in 2007 recommended surgery before age 1 year [20]. Based on this recommendation we decided from 2008 to operate all boys at 9 months of age.

Furthermore we ceased to recruit boys with unilateral palpable cryptorchidism into the study due to the fact that we had a considerable number of boys with this type of cryptorchidism enrolled. Recruitment only continued in boys with less
frequent phenotypes; non-palpable and bilateral cases. In summary these measures lead to a skewed distribution in the patient material in bilateral, intraabdominal and monorchid groups.
4. Patient material

The different subgroups and number of included boys are shown in table 2. In study I to IV the study populations varied as presented in each study.

Table 2. The total number of included and categorized boys.

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral palp. treated at 9 mo.</td>
<td>78</td>
</tr>
<tr>
<td>Unilateral palp. treated at 3 y.</td>
<td>85</td>
</tr>
<tr>
<td>Bilateral palp. treated at 9 mo.</td>
<td>28</td>
</tr>
<tr>
<td>Bilateral palpable treated at 3 y.</td>
<td>8</td>
</tr>
<tr>
<td>Intraabdominal treated at 9 mo.</td>
<td>20</td>
</tr>
<tr>
<td>Intraabdominal treated at 3 y.</td>
<td>15</td>
</tr>
<tr>
<td>Monorchid treated at 9 mo.</td>
<td>27</td>
</tr>
<tr>
<td>Monorchid treated at 3 y.</td>
<td>17</td>
</tr>
<tr>
<td>Spontaneous descent unilateral</td>
<td>71</td>
</tr>
<tr>
<td>Ascending (recurrent)</td>
<td>20</td>
</tr>
<tr>
<td>Mixed</td>
<td>57</td>
</tr>
</tbody>
</table>

4.1 Study I

Boys with unilateral palpable undescended testes randomized to surgery at 9 months (n=70) of age were examined from birth (0-3 weeks) until 2 years of age with longitudinal ultrasound measurements of testicular volume and compared to
yet non-treated boys (n=79) with unilateral palpable undescended testes also followed until 2 years of age.

4.2 Study II

Boys with unilateral palpable undescended testes randomized to surgery at 9 months (n=72) and 3 years (n=83) of age were examined from birth (0-3 weeks) and until 4 years of age with longitudinal ultrasound measurements of testicular volume.

4.3 Study III

Boys with spontaneous unilateral descent (n=71) were examined from birth (0-3 weeks) and until five years of age with longitudinal ultrasound measurements of testicular volume and compared to boys with unilateral palpable testes that were operated on at age 9 months (n=78) and 3 years (n=85) of age. Boys with initial spontaneous descent followed by ascense (n=20) were included.

4.4 Study IV

The patient material in study IV included boys with spontaneous descent, unilateral and bilateral cryptorchidism. The groups were investigated regarding morphological, volumetric and endocrine data.

Morphometric part

A total of 213 biopsies from 215 boys were collected and testicular volume was measured at time of surgery at 9 months or 3 years of age.
Table 3. The number of biopsies obtained in boys treated at 9 months and at 3 years is shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>Biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral palpable</td>
<td></td>
</tr>
<tr>
<td>Surg. 9 mo.</td>
<td>73</td>
</tr>
<tr>
<td>Surg. 3 y.</td>
<td>74</td>
</tr>
<tr>
<td>Bilateral palpable</td>
<td></td>
</tr>
<tr>
<td>Surg. 9 mo.</td>
<td>27</td>
</tr>
<tr>
<td>Surg. 3 y.</td>
<td>6</td>
</tr>
<tr>
<td>Intra-abdominal</td>
<td></td>
</tr>
<tr>
<td>Surg. 9 mo.</td>
<td>21</td>
</tr>
<tr>
<td>Surg. 3 y.</td>
<td>12</td>
</tr>
</tbody>
</table>

A number of boys with bilateral cryptorchidism were as discussed before included in the mixed group due to the fact that both sides could not be surgically corrected at the same occasion, often one side was corrected at 9 months and the second side was treated later, thus resulting in a inhomogeneous phenotype and therefore the boys were added to the mixed group. However in the biopsy study some of these boys were included since the biopsies represent data from boys with bilateral cryptorchidism at 9 months of age, even though the other contralateral side was operated on at another age.

Endocrine part

Blood samples for analysis of inhibin B and FSH levels were collected at ages 0-3 weeks, 2 and 6 months and 1, 2 and 4 years of age. LH and testosterone were only analyzed at ages 0-3 weeks, 2 and 6 months since blood levels at following ages were very low and often not measurable. To investigate the relationship between inhibin B and FSH all measurements from all groups were added together at all time points.
The boys were grouped and blood sampling was performed according to the following: Preoperative (not yet operated on) and postoperative boys (operated on) with unilateral palpable and non-palpable intraabdominal and monorchid undescended testes. Boys with bilateral palpable undescended testes at pre- and postoperative ages. Boys with spontaneous descent and all boys lumped together.

Table 4. Table shows the different subgroups and ages at blood sampling.

<table>
<thead>
<tr>
<th>Group</th>
<th>Ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>0-3 weeks, 2 and 6 mo. and 1 and 2 y.</td>
</tr>
<tr>
<td>Postoperative</td>
<td>1, 2 and 4 years</td>
</tr>
<tr>
<td>Bilateral</td>
<td>0-3 weeks, 2 and 6 mo. and 4 years</td>
</tr>
<tr>
<td>Spontaneous descent</td>
<td>0-3 weeks, 2 and 6 mo. and 1, 2 and 4 y.</td>
</tr>
<tr>
<td>All groups</td>
<td>0-3 weeks, 2 and 6 mo. and 1, 2 and 4 y.</td>
</tr>
</tbody>
</table>

Volumetric part

Ultrasound measurements from patients from groups palpable unilateral, bilateral and non-palpable unilateral intraabdominal testes were studied from birth and up to five years of age and correlated to ruler measurements obtained at time of surgery.
5. Methods

5.1 Surgical methods

The surgical methods used in our series of patients included a standardized open single-staged inguinal method for all palpable testes and non-palpable intraabdominal testes after preceding diagnostic laparoscopy.

In our series we did not use the single-incision transscrotal approach even though some testes were suitable for the use of this method, the reason for this was that we wished to standardize the procedure.

A Fowler-Stephens open single-step procedure (as described before) with division of the vessels was done in a few cases where sufficient length to scrotum could not be achieved. An inguinal retroperitoneal dissection was always tried before the Fowler-Stephens procedure was done.

An initial diagnostic laparoscopy was performed in boys with non-palpable testes, in some cases an atrophic testis was removed during laparoscopy or through an inguinal approach. The same surgeon (CK) performed more than 95 % of all the surgical procedures.

Open inguinal approach

A transverse incision in the groin 1-2 cm proximal to the pubic bone was done (over the inguinal ring), in some cases with intraabdominal testes the incision was extended laterally and/or placed more proximally.
The superficial fascia (Scarpa) was incised and the external oblique aponeurosis was identified. If necessary the testis was then freed distally from the gubernaculum attachment and proximally around the external ring.

The extern oblique aponeurosis was incised in the direction of the spermatic vessels approximately 1-3 cm depending on the need, caution being made to the ileoinguinal nerve. Further blunt and sharp dissection around the spermatic cord and proximally around the internal ring was done.

The tunica vaginalis was then opened and the testis, epididymis, vessels, vas deferens and other abnormalities were described. In many cases a hydatid of Morgagni was identified and removed. Thereafter the testis was measured with a ruler (length, width, height).

The spermatic vessels and the vas deferens located in the retroperitoneal space were carefully dissected away from the peritoneum. The hernial sac was not ligated at this point; it was kept clamped and lifted thus facilitating the access to the retroperitoneal space. If the retroperitoneal space was not clearly identified optimal high dissection was harder to achieve.

In cases of intraabdominal testes a blunt and sharp retroperitoneal dissection of tissues surrounding the spermatic vessels was done, the higher the location of the testis the more extensive and proximal dissection. This step of the surgical procedure is sometimes difficult and crucial for achieving optimal length of the vessel for the testis to be placed in the scrotum without the need of a Fowler-Stephens procedure.
Next the hernial sac was ligated. Additional length of a few mm of the vessels was sometimes achieved by the transposition of vas deferens and spermatic vessels under inguinal vessels.

The orchidopexy was achieved by creating a subdartos pouch for the testis in the scrotum. This was done by a transversal scrotal skin incision, about 1 cm in length, followed by a blunt dissection both proximally (to prevent an upward traction of the scrotal skin) and distally under the scrotal skin from the incision. Next the subcutaneous scrotal fascia was incised where a suture was placed un-knotted in the “corner” of the dartos fascia.

A passage or tunnel was then bluntly created with the use of the index finger from the inguinal incision down to the scrotal incision. A surgical clamp was then inserted through the scrotal incision touching the index finger; this position was kept while retracting the surgical clamp towards the inguinal incision simultaneously withdrawing the finger, still keeping the contact between the finger and the instrument. The testis was then placed and fixed in the surgical clamp by holding the testis in the epididymal ligament, this was followed by a retraction of the clamp with the testis through the passage back to the scrotal incision.

The previously placed resorbable unligated suture was used to narrow the width of the dartos fascia and finally ligated to prevent the testis from retracting to a retained position. Sutures in the capsule of the testis for fixation were avoided at all times. Finally the skin incisions, external aponeurosis, subcutaneous tissue was closed using resorbable sutures. According to the study design a testicular biopsy was taken before the testis was placed in the subdartos pouch.
So in summary, scrotal position for the palpable testes is often easy to achieve. The surgical challenge to accomplish this is for the intraabdominal testes. The identification of the retroperitoneal space by the lifting of the peritoneum and the subsequent high dissection of the vessels is crucial for achieving sufficient length.

Fig.6. Series of pictures illustrating some of the different steps in an open single-staged scrotal orchidopexy
for the intraabdominal testes without dividing the vessels thus avoiding a Fowler-Stephens procedure.

*Fowler-Stephens procedure*

If sufficient length could not be achieved after extensive retroperitoneal dissection a single-step open Fowler-Stephens procedure was performed by dividing the spermatic vessels proximally in most cases.

*Laparoscopic procedure*

In all boys with non-palpable testes an initial diagnostic laparoscopy was performed. After an umbilical incision according to Hasson a trocar was inserted and carbon dioxide was insufflated with a pressure of 10 mm Hg and a low rate of 1 l/minute. After completed insufflation a 0 or 30 degree 5 mm laparoscope was inserted, if required additional working trocars were inserted. The position and size of the testis, the internal rings, contralateral side and abnormalities were described. The next surgical step was decided upon after assessment of the intra-operative findings according to the algorithm below.
Fig. 7. Decision tree for the non-palpable intraabdominal testis at laparoscopy.
5.2 Testicular biopsies

A total of 213 biopsies collected from 215 boys biopsies were analyzed. An open wedge of tissue approximately 1x2 mm in size was removed from the undescended testis after incision of the capsule. In most cases the biopsy was taken with a pair of scissors however if the testicular tissue was not “bulging” as seen in atrophic testes forceps were used. The capsule was closed with a 6:0 resorbable suture after the biopsy was taken.

For microscopy the biopsies were fixed by immersion in 5% glutaraldehyde dissolved in s-collidine buffer (0.16 M, pH 7.4) at room temperature as described earlier [140]. Thereafter, the tissues were embedded in epoxy resin (Glycidether 100, Merck, Germany), cut into semi-thin (1-μm) sections and stained with toluidine blue and periodic acid-Schiff (PAS) staining (PAS-kit 1016460001, Merck, Germany). In each round cross-section of the seminiferous cord, cells exhibiting nuclear morphology typical of germ cell as well as Sertoli cells were counted. The cord diameter and the proportion of the testis made up by the interstitial compartment were determined with a morphometric program (Leica IM500 Version: 4.0, Leica Microsystems Imaging Solutions Ltd., Cambridge, UK).

5.3 Testicular volume

The testicular volume of the retained and scrotal testes was determined with ultrasound measurements at the ages of 0-3 weeks, 6 months, and yearly up to 5 years of age, performed by radiologists.

Two formulas for a prolapsed ellipsoid have been used, one for the ultrasound measurements and another formula for the ruler measurements. The reason for this is that the radiologist in study I-III used a different formula to calculate the
testicular volume than the one used for the ruler measurements; in spite of this fact both formulas yield the same results.

The ultrasound examinations were performed using high-resolution scanners. Testicular volumes were calculated using the approximation for a prolapsed ellipsoid: \( V = \frac{\pi}{6} \times L \) (length) \( \times W \) (width) \( \times H \) (height). One experienced radiologist performed the majority of ultrasound procedures (UH). All examiners were blinded for previous results and preliminary data. However, scars following the orchidopexies were obvious in most cases.

The measurements taken at time of surgery were done using a ruler and the volume was calculated using the following formula: \( V = \frac{4}{3}\pi a b c \). where \( \pi=3.14 \), and \( a, b, c \) are the semi-axes of an ellipsoid. The testicular volume was assessed prior to the biopsy. The same pediatric surgeon (CK) performed more than 95% of all the ruler measurements.

5.4 Hormonal assays

FSH and LH assays

Serum FSH and LH were measured in singlicate with commercially available reagents from Perkin Elmer/Wallac (article-nr B017-201) on a 1235 AutoDELFIA immunoassay system. For more details on the methodology see original paper IV.

Inhibin B assay

Inhibin B was measured in duplicate in serum with, at the time, commercially available ELISA-reagents from Oxford Bio-Innovation. For more details on the methodology see original paper IV and the methodology described by Groome et al [141].
Testosterone assay

Testosterone was measured in duplicate in serum with commercially available reagents from Orion Diagnostica as modified and optimized according to Ankarberg and Norjavaara [142].

5.5 Statistical methods

All used methods and analyzes were done by statisticians and described in more detail in the original papers I to IV.
6. Results

6.1 Study I

Testicular volume was determined with ultrasound measurements at ages 0-3 weeks, 6 months, 1 and 2 years of age obtained from boys with palpable unilateral cryptorchidism randomized to surgery at 9 months (n=70) or at 3 years (n=79) of age.

*Development of the scrotal testes from birth up to two years of age (Fig. 8)*

Between 0-3 weeks and 6 months of age the normally descended testis increased significantly in median volume (p<0.001), whereas from 6 to 24 month there was no further increase in either group (treated and non-treated) as shown in figure 8:
Fig. 8. Development of testicular volume of the scrotal testes in boys with palpable unilaterally undescended testes. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=70) or 3 years (n=79). The number of performed ultrasonographies is shown in the figure (Medians and 95% confidence intervals).

*Development of the retained testes from birth to two years of age (Fig. 9 & 10)*

Between 0-3 weeks and 6 months of age the retained testes increased in median volume (p<0.001). At both 1 and 2 years of age there was a significant difference in median volume between the treated and the non-treated group (p<0.01, p<0.001 respectively) as shown in figure 9:
Fig. 9 Development of testicular volume of the **retained** testes in boys with palpable unilaterally undescended testes from age 0-3 week to 2 years. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=70) or at 3 years (n=79). The number of performed ultrasonographies is shown in the figure (Medians and 95% confidence intervals).

The ratio between the scrotal and retained testes provides a comparison of the degree of growth deficit in the retained testes. In the treated group the median ratio increased from 6 months to 2 years (p<0.01) whereas in the untreated group the ratio decreased. Furthermore at age 2 years there was a difference in ratio between the treated and the untreated group (p<0.001).
Fig. 10. Development of the ratio between the retained and scrotal testes. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=70) or 3 years (n=79). The number of performed ultrasonographies is shown in the figure. (Medians and 95% confidence intervals).

Interestingly already at birth there was a significant difference in median volume between the retained and scrotal testes. In order to investigate what effect the suprascrotal position had on the retained testes we extrapolated a group of boys (n=22) that had almost equal size of the retained testes compared to the scrotal testes at birth. Results showed that at 6 months the retained testes had grown less than the scrotal ones (p<0.001), clearly indicating that the suprascrotal position plays a significant role for testicular growth.
6.1.1 Conclusion

Surgical treatment at 9 months of age resulted in a significant catch-up testicular growth from 6 months to 2 years compared to non-treatment. Furthermore we have demonstrated that prenatal factors play a role for testicular volume at birth but also that the supracrotal environment has an adverse effect on testicular growth.

6.2 Study II

This study is a follow-up from study I where surgical treatment at 9 months were compared to non-treatment up to 2 years of age. In this study we continued to analyze the testicular volume up to 4 years of age from boys with palpable unilateral cryptorchidism randomized to surgery at 9 months (n=72) or at 3 years (n=83).

Development of the scrotal testes from birth up to four years of age (Fig. 11)

In accordance with results from study I the scrotal testes grew significantly from birth to 6 months of age, with no subsequent growth up to two years, however a growth from 6 months to 4 years was demonstrated (p<0.001), no difference was observed in testicular volume of the scrotal testes between the randomized groups at any age as shown in figure 11:
Fig. 11. Development of testicular volume of the scrotal testes in boys from age 0-3 week to 4 years. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=72) or at 3 years (n=83). The numbers of testes examined are shown in the figure. (Medians and 95% confidence intervals).

Development of the retained testes from birth to four years of age (Fig. 12 & 13)

The retained testes grew significantly from birth to 6 months of age, furthermore there were significant differences between the scrotal and previously retained testes at all ages (p<0.001) up to 4 years of age in both groups.

Surgical treatment at 9 months resulted in a significant growth (p<0.001) from 6 months to 2, 3 and 4 years of age whereas in the group treated at 3 years there was no significant growth before and after orchidopexy.
In the group that underwent surgery at 9 months there was an increase in the ratio from 6 months to 4 years (p<0.001) whereas a decrease in the ratio during the same period was observed in the group that was treated at 3 years (p<0.001). There was a significant difference in testicular volume and ratios at ages 2, 3 and 4 between the two groups (p<0.001) as shown in figure 12 and 13:

Fig.12. Development of testicular volume of the initially retained testes in boys from age 0-3 weeks to 4 years. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=72) or at 3 years (n=83). The number of testes examined is shown in the figure (Medians and 95% confidence intervals).
Fig. 13. Development of the ratio between the retained and scrotal testes in boys from age 0-3 weeks to 2 years. At 6 months of age all boys were randomised to orchidopexy at 9 months (n=72) or 3 years (n=83). The numbers of performed ultrasonographies are shown in the figure (Medians and 95% confidence intervals).

### 6.2.1 Conclusion

We have shown that the partial catch-up growth of initially retained testes continued up to 4 years in boys who were operated on at 9 months. However, the testicular volumes were still not equal to their scrotal counterparts. On the other hand, in the group treated later the testes showed a decrease in growth compared to their scrotal counterparts and a significant difference in testicular volume and ratio compared to all ages in the early treated group.
6.3 Study III

Volumetric data from birth up to 5 years of age were obtained from 71 boys with spontaneous descent that at birth had palpable unilaterally cryptorchid testes. These boys were compared to boys that were still unilaterally cryptorchid and randomized to surgery at either 9 months (n=78) or 3 years (n=85) of age.

Age at spontaneous descent and ascending testes

91 boys showed spontaneous descent after being initially cryptorchid, 71 testes remained descended whereas 20 ascended to a cryptorchid position (22 %). Result showed that spontaneous descent occurred mainly before 2 months of age (82 %). The recurrences occurred evenly distributed between 1 and 5 years of age.

Growth of the spontaneously descended testes compared to surgically treated testes and their scrotal counterparts (Fig. 14)

The testicular volume of the scrotal testes did not differ between any of the groups at any time point.

At inclusion age 0-3 weeks the volume of the retained or the spontaneously descended did not significantly differ between any of the groups, although at 6 months the group with spontaneous descent showed the highest mean volume compared to the yet non-treated groups.

In study I and II we concluded that the surgically treated testes were significantly larger than their scrotal counterparts at all ages up to 4 years of age, this study has demonstrated that the difference still persists up to 5 years of age, also showed for the spontaneously descended testes compared to their scrotal counterparts at all ages (p<0.001), except at 6 months.
In study II we demonstrated that the retained testes between the early and late treated group showed significant differences in testicular volume up to 4 years of age, in this study we conclude that the significant difference still persists up to 5 years of age (p<0.023).

When comparing the spontaneously retained testes to the surgically treated testes we observed no difference between the spontaneously descended testes and the ones treated at 9 months, however when compared to testes treated at 3 years the spontaneously descended testes were larger at all ages (except at 0-3 weeks), with significant differences at ages 2, 4 and 5 years.

![Figure 14. Cross sectional data: The testicular volumes of the retained (open circles) and scrotal (filled circles) testes from birth until 5 years of age. From left to right: early treated group (9 month), late treated group (3 years) and the spontaneously descended group. Means and 95 % confidence intervals are shown.](image-url)
Comparing the ratios between the three groups from birth to 5 years of age (Fig. 15)

The ratio provides a direct comparison of growth between the retained and scrotal testis and thus an index of the degree of growth deficit of the retained or initially retained testes.

The group surgically treated at 9 months showed an increased ratio from 6 months to 5 years (p<0.013), whereas the group treated at 3 years decreased its ratio over the study period, however not significant. Interestingly the group with spontaneous descent also showed a decreasing ratio over the study period (p<0.021).

When comparing groups, there was a significant difference in the ratio over the study period between the early treated (9 months) and late treated (3 years) group (p<0.003) and also with the group with spontaneous descent (p<0.001).
6.3.1 Conclusion

We have demonstrated that spontaneously descended testes show a significantly impaired growth from birth up to 5 years of age compared to their scrotal counterparts and that they also are more prone to subsequent ascent (recurrent undescended testes). Orchidopexy at 9 months of age improved the testicular growth up to 5 years compared to those treated at 3 years, although these testes did not fully reach the growth rate of the scrotal testes in any group. Also, early surgery improved testicular growth of the previously retained testes up to 5 years compared to their scrotal counterparts (ratio). In addition, the growth was significantly higher than in the group treated later and, interestingly, than in the
group with spontaneous descent. The smallest testes were the ones treated late whereas there was no significant difference in testicular volume between the testes operated on at 9 months compared to the spontaneously descended ones. Over time, however, the spontaneously descended testes grew less (decreased ratio) compared to the testes treated at 9 months.

6.4 Study IV

Morphometric (biopsies) and volumetric data (ruler measurements) were collected from boys with palpable unilateral, bilateral and intraabdominal testes surgically treated at 9 months or 3 years of age.

Blood samples for analysis of inhibin B and FSH were collected from boys with unilateral or bilateral cryptorchidism before and after surgical treatment and from boys with spontaneous descent at ages 0-3 weeks, 2 and 6 months, 1, 2 and 4 years. Testosterone and LH levels are reported for ages 0-3 weeks, 2 and 6 months only, since blood levels at subsequent ages were very low and often not measurable. To investigate the relationship between inhibin B and FSH all measurements from all groups were added together at all time points.

Morphometric and volumetric results (Table 5)

All testes treated at 9 months showed significantly more germ and Sertoli cells 100/cords, greater testicular volume, greater diameter of seminiferous tubules and less interstitial tissue when compared to those treated at 3 years (p<0.001).

When comparing the early (9 months) treated palpable unilateral, bilateral and intraabdominal groups to each other the only observed significance was in the number of Sertoli cells between the unilateral and intraabdominal groups.
At 3 years the same correlations showed that the only observed significance (borderline) was in the number of germ cells between the palpable unilateral and intraabdominal group, thus indicating that the late treated intraabdominal testes showed the worst germ cell depletion of all groups (both early and late groups).

Correlation between testicular volumes and the number of germ and Sertoli cells/100 cords were evaluated for all the cryptorchid boys lumped together and the testicular volume at surgery correlated significantly to the number of both germ and Sertoli cells (p<0.001).

At 9 months the same significant correlations were found for germ and Sertoli cells (p<0.001 and p<0.01 respectively), but at 3 years, the correlation was significant (p<0.05) for Sertoli cells only, however at 3 years there were very few germ cells and therefore statistical difference was difficult to show.
Table 5: Testicular volume (Vol. (cm³)), cord diameters (Diam. (µm)), percentage of interstitial tissue (Int. Tissue (%)) as well as counted numbers of Sertoli cells per 100 seminiferous cords (SC/100 sc (n)) and germ cells per 100 seminiferous cords (GC/100 sc (n)) were counted and are listed for unilateral, bilateral and intraabdominal cases (G). Number of samples (n) for cord diameter, percentages of interstitial tissue, Sertoli cells and germ cells per 100 seminiferous cords are given in the first column, whereas the numbers of samples for volume measurements are stated together with the volume.

<table>
<thead>
<tr>
<th>Sample (n)</th>
<th>Vol. (ml) (n)</th>
<th>Diam. (µm)</th>
<th>Int. tissue (%)</th>
<th>SC/100 sc (n)</th>
<th>GC/100 sc (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral 9 month (73)</td>
<td>0.5 +/- 0.2 (74)</td>
<td>56.2 +/- 7.1</td>
<td>46.6 +/- 8.5</td>
<td>3076.3 +/- 496.2</td>
<td>96.1 +/- 68.7</td>
</tr>
<tr>
<td>Bilateral 9 month (27)</td>
<td>0.4 +/- 0.2 (31)</td>
<td>53.8 +/- 7.2</td>
<td>47.4 +/- 7.2</td>
<td>2850.6 +/- 537.6</td>
<td>69.7 +/- 49.2</td>
</tr>
<tr>
<td>Intraabdominal 9 month (21)</td>
<td>0.4 +/- 0.2 (22)</td>
<td>54.1 +/- 8.0</td>
<td>49.2 +/- 12.7</td>
<td>2784.1 +/- 532.0</td>
<td>106.5 +/- 79.0</td>
</tr>
<tr>
<td>Unilateral 3 years (74)</td>
<td>0.4 +/- 0.2 (74)</td>
<td>41.6 +/- 3.9</td>
<td>68.8 +/- 10.8</td>
<td>2118.2 +/- 338.4</td>
<td>9.8 +/- 21.3</td>
</tr>
<tr>
<td>Bilateral 3 years (6)</td>
<td>0.3 +/- 0.1 (8)</td>
<td>43.7 +/- 6.0</td>
<td>60.1 +/- 8.1</td>
<td>2364.6 +/- 383.1</td>
<td>10.2 +/- 13.1</td>
</tr>
<tr>
<td>Intraabdominal 3 years (12)</td>
<td>0.3 +/- 0.2 (10)</td>
<td>40.1 +/- 2.3</td>
<td>71.1 +/- 10.1</td>
<td>2105.5 +/- 407.4</td>
<td>2.2 +/- 3.4</td>
</tr>
</tbody>
</table>

Cross sectional pictures are shown to illustrate the morphometric findings in palpable and non-palpable testes from boys treated at 9 months and at 3 years of age (fig 16-18).
Fig. 16. Testicular histology of cryptorchid testes. Cross sections the seminiferous cords from 9 month (A, C and E) and 3 year (B, D and F) old patients are shown after PAS staining. Areas shown with a higher magnification are marked with a square (A-F) and are depicted on the left and right side of the panel (a-f). Germ cells with normal nuclei (white arrowheads) as well as multi-nuclei (black arrowheads) were observed in unilateral (A and B), bilateral (C and D) testicular biopsies from 9 month and three year old patients, as well as intra-abdominal cases of 9 month old patients. Only few germ cells were observed in intra-abdominal cases of 3 years old boys (F). Scale bars: 50µm.
Fig. 17: Cross sections of cryptorchid testes taken with light microscopy. A, early unilateral; B, late intra-abdominal. Germ cells (white arrowheads: gonocytes and spermatogonia) are shown in 9 month old testis (A), but are nearly fully depleted in 3 year old testis (B). Cell degeneration (yellow stars) can be observed in 3 year old testis (B).
Hormonal results (Fig. 19 a-e)

All analyzed hormones showed a peak in serum levels during the first two months of life, thereafter decreasing to lower levels throughout the study periods. LH and testosterone did not differ between any of the groups at any of the time points (Fig. 19 c-d).
At age 2 months the group with bilateral cryptorchidism showed higher inhibin B serum levels than the groups with spontaneous descent and the boys that were still cryptorchid (p<0.001 and p<0.008 respectively), however at age 4 years the group with spontaneous descent showed higher inhibin B levels than the previously cryptorchid boys (p<0.008). Overall FSH and inhibin B levels decreased over time in a similar fashion (Fig. 19 e).

At 2 years of age, the boys that had been operated on at 9 months had significantly lower FSH levels than those that had not yet been treated, and also higher inhibin B values, however not significant (fig 19 a-b). Furthermore, neither of the hormones analyzed at 0-3 weeks or 2 months could predict spontaneous descent.

**Correlations morphology, testicular volume and hormones**

Correlations between inhibin B levels at 6 months and at 4 years (before and after orchidopexy) and testicular volume were also investigated. For the boys with unilateral cryptorchidism inhibin B values at both 6 months and 4 years correlated positively with testicular volume of the retained testes at 4 years (p<0.005 and p<0.0014 respectively). On the contrary boys with bilaterally undescended testes, testicular volume at 6 months correlated negatively with inhibin B values at 4 years, i.e. boys with small testes had high inhibin B values at 4 years.

Furthermore correlations between hormones taken at 2 months and the number of germ and Sertoli cells were investigated to assess whether any of the hormones taken during minipuberty can predict future spermatogenesis. The only positive correlation found was between inhibin B values at 2 months for the boys with unilaterally palpable testes with the number of Sertoli cells at 9 months (p<0.001) but not at 3 years. No other correlations were identified between any of the hormones for any of the groups at neither 9 months nor 3 years.
Fig. 19 a-e. Hormone levels for the different patient groups (spontaneous descent, preoperative unilateral, postoperative unilateral and bilateral groups). Inhibin B and FSH was measured at ages 0-3 weeks, 2, 6, 12, 24 and 48 months (a-b) and LH and testosterone was measured at ages 0-3 weeks, 2 and 6 months (b-c). Inhibin and FSH levels for all boys are shown in graph e.
6.4.1 Conclusion

The morphometric data show that at 9 months, the undescended testes have a significantly larger number of germ and Sertoli cells, greater diameter of seminiferous tubules and a higher ratio interstitial/tubular tissue than at 3 years of age. Furthermore, at 3 years of age the intraabdominal testes showed the worst depletion of germ cells, clearly indicating that the longer the testes reside in this position the higher risk for impaired spermatogenesis.

The above mentioned morphometric data are in concordance with previously published volumetric studies (study I and II), thus suggesting that early surgery is beneficial for spermatogenesis and giving further support for early orchidopexy.

Furthermore we have in this study proved that ultrasound measurements of testicular volume correlate with ruler measurements at the time of orchidopexy and that the ruler measurements significantly correlate with the number of germ and Sertoli cells. To our knowledge this has never been proven in cryptorchid boys prior to this study, but it clearly indicates that testicular volume can be used as a proxy for testicular function in cryptorchid boys.

Serum levels of hormones reflect the output of both testes and are therefore not easily interpreted in unilateral cryptorchidism, which account for a majority of the cryptorchid boys. However, inhibin B may be of interest in bilateral cryptorchidism, where one would expect lower values, reflecting poor Sertoli cell function. As discussed before in the section on fertility inhibin B may possibly serve as a marker for compromised paternity in men with a history of bilateral cryptorchidism, whereas for cryptorchid boys the results are contradictive. Therefore the predictive value of prepubertal inhibin B for the quality of spermatogenesis in adulthood remains to be established.
In our study we surprisingly found that the levels of inhibin B during minipuberty were actually the highest in the bilateral group compared to unilateral cases and boys with spontaneous descent (presumably the most normal testes). In addition FSH levels were high in the bilateral cases at this age. This was reversed at the age of four when the group with spontaneous descent showed higher inhibin B and FSH levels than the previously cryptorchid boys. We find it difficult to interpret these data and therefore no conclusions can be drawn.

Furthermore we have shown that inhibin B correlated positively with FSH levels throughout the study period. We have also demonstrated that boys with unilateral cryptorchidism and high levels of inhibin B at 6 months and 4 years also had bigger testes at 4 years than boys with low levels, thus indicating that inhibin B taken at 6 months can be used as a marker for testicular growth. However, we have not been able to show the same in bilateral cases, so the results are inconclusive and remain highly speculative.

In summary, we have not been able to show that any of the hormones taken during the first months of life can serve as a marker for future spermatogenesis or to predict spontaneous descent of the testes.

### 6.5 Surgical results

**Success rate by position and age**

Success rates for palpable uni- and bilateral cases treated at 9 months and 3 years were 95 % (5 atrophies/109 procedures) and 98 % (2/83) respectively, with an overall rate of 96 % (7/192).

Success rates for intraabdominal testes treated at 9 months and 3 years were 83 % (4/24) and 100 % (0/12) respectively with an overall rate of 90 % (4/36).
Success rates at 9 months vs. boys treated at 3 years (both palpable and non-palpable were 93 % (9/133) and 98 % (2/95) respectively.

**Success rate by procedure**

Success rate for the open inguinal approach was 95 % (11/227). Three open single-staged Fowler-Stephens procedures were performed, two of them showed atrophy at follow-up.

Recurrences occurred in three cases. In one of these cases an orchidectomy was done, and in total three orchidectomies were undertaken in the study.

**6.5.1 Conclusion**

We have demonstrated that the open inguinal approach for both palpable and intraabdominal testes is an efficient (95 %) surgical method for achieving scrotal position without atrophy. By the use of this method with extensive retroperitoneal dissection (when needed) most testes could be brought down to scrotum without a Fowler-Stephens procedure and with very few recurrences.

To our knowledge there are no previously published randomized studies on success rate correlated with the age at orchidopexy. In our series the overall success rate of orchidopexy for all testes (both palpable and intraabdominal) at 9 months and 3 years was 93 % and 98 % respectively.
7. Discussion

Cryptorchidism is the most common anomaly in newborn boys [3]. Despite its frequency there are many controversies regarding several aspects, including aetiology, mode and timing of treatment as well as the risk of infertility and testicular cancer. Almost all controversies are the result of a lack of prospective long-term studies with clear definitions and nomenclature; consequently the numerous studies very often include a mix of phenotypes thus showing contradictive results. Furthermore many studies are conducted on adult men with a history of cryptorchidism whereas others are conducted on children with no follow-up throughout puberty and into adulthood. Decades ago, treatment was generally given at a higher age than is currently recommended, which makes it very difficult to assess, on the basis of published studies, whether surgery in infancy improves spermatogenesis.

The main objectives of this thesis report are: to present the first prospective randomized study on the timing of treatment, to identify markers of testicular function important to future spermatogenesis, and to study the growth and natural course of spontaneously descended testes.

In our studies we diagnose undescended testes by using a clear definition and nomenclature for the different forms of cryptorchidism, which have resulted in several (nine) subgroups of cryptorchid boys including a mixed group with inhomogeneous phenotypes. In addition, we also follow boys with spontaneous descent and with recurrent cryptorchidism, which to our knowledge has never before been done prospectively from birth.

At the start of the study in 1998 boys with congenital undescended testes were randomized to surgery at either 9 months or 3 years of age. In 2008 the randomization was discontinued since the Nordic consensus group, partly on the
basis of our published volumetric studies, made the recommendation that surgery should be done before the age of one year [20, 143].

The discontinuation of the randomization led to a skewed distribution of boys in non-palpable and bilateral cases, with a higher number of boys treated at nine months of age compared to three years of age. Nonetheless the design of the study and number of boys included has provided us with a unique opportunity to evaluate the success of treatment at different ages in the different forms of cryptorchidism.

The optimal timing of surgery has long been debated. Based on indirect proof from morphological studies the general consensus is that treatment should be offered early in childhood [100-102, 109-111, 138, 139]. However, this recommendation has prior to our study not been proven in prospective randomized studies where boys actually have been randomized to surgery at different ages and then followed throughout puberty and into adulthood.

Some of the cited morphological studies suggest that treatment in early infancy is beneficial compared to later treatment but since they are retrospective there is usually no clear definition and nomenclature of the diagnosis. Furthermore, other important markers for testicular function like testicular size and serum levels of hormones have not been analyzed and compared to the morphology of the testis.

In this study we have shown that surgical treatment at nine months of age is beneficial and results in a significant difference in the number of germs and Sertoli cells, greater testicular volume and diameter of the seminiferous cords and less interstitial tissue than we find in boys treated at three years of age, all of which clearly supports early orchidopexy.
Moreover, these morphometric results agree with our previously published studies on testicular volume. They clearly demonstrate that testes orchidopexied at nine months show a significant partial catch-up growth of the previously retained testes up to five years of age compared to testes in boys treated at three years of age, where no such catch-up growth can be observed [143, 144].

However, one can argue that between nine months and three years of age there is a decrease in both germ and Sertoli cells in normal testes [23, 109]. Therefore our findings with a decreased number of cells do not prove that treatment at nine months is preferable to three years. However, the magnitude of the decrease is influenced by early treatment.

In a study by Paniagua et al. on post-mortem boys it was shown that the reduction of germ cells for boys 0 - 1 year compared to 3 - 4 year old boys was approximately 5% for spermatogonia and for Sertoli cells 15% [23]. The ages at orchidopexy in our study are fully comparable to the post-mortem ages and our results demonstrate a reduction between nine months and three years of approximately 67 % (germ cells) and 25 % (Sertoli cells) respectively, thus clearly indicating that cryptorchid testes suffer more over time compared to normal testes.

As discussed before, studies on adult men have indicated that testicular volume correlates with testicular function, but prior to this study this has never been shown in cryptorchid boys [24, 97, 130, 131]. Here, we have been able to demonstrate a significant correlation between measurements of testicular volume taken at the time of surgery and the number of spermatogonia and Sertoli cells. This proves that testicular volume correlates to spermatogenesis in cryptorchid boys and consequently that it can be used as a marker for testicular function. If the difference in testicular size between early and later treated boys persists throughout puberty and into adulthood remains to be proven in future studies.
In summary, early orchidopexy in infancy seems to be favourable to future spermatogenesis, but the data on testicular volume show that even though partial catch-up growth occurs in the early treated boys, the retained testes do not fully reach the size of scrotal testes.

One limitation of this study is that we did not have a control group for testicular volume, and we were unable to find studies of normal testicular growth that could be used as a reference material in the literature. Therefore the contralateral scrotal testes in the unilaterally cryptorchid boys were used as a reference of normal testes since they did not differ in size from any of the groups, including the scrotal testes in boys with spontaneous descent.

We have shown that already at birth there is a significant difference in testicular volume between the retained and scrotal testes, indicating that prenatal factors play a role. Furthermore, we have demonstrated that the longer the testes reside in a retained position the less the testicular growth, thus clearly showing that the position in the groin has an adverse effect on testicular development. In support of this conclusion, other studies indicate that the elevated temperature in the groin has adverse effects on testicular development [115, 118, 145].

However, since the first ultrasound examination in our series was done during the first three weeks of infancy, it is possible that the testes were normal at birth but thereafter grew less than their scrotal counterparts resulting in a smaller volume at the time of the first ultrasound examination.

We hypothesize that the cryptorchid position with subsequent higher temperature is more likely to cause adverse effects if the testis is in an “active mode” compared to a cryptorchid position later in childhood when the testis is dormant. We know from published volumetric results in studies I and II that the growth from birth to six months of age, of both retained and scrotal testes is more substantial compared
to other time periods in the study. In other words, our hypothesis is that the testicular position during the first few months of life is crucial to testicular growth. This is supported by the fact that the substantial growth during this period coincides with peak levels of gonadotrophins (the “mini-puberty”), of importance for testicular growth.

The difference in testicular volume already at birth is interesting and raises questions: Can testicular growth be improved further by treating cryptorchid boys even earlier than at nine months of age? If so, how soon in infancy would treatment be required for the previously retained testes to catch up with the size of their scrotal counterparts? Maybe, the latter is not feasible if these changes have already occurred prenatally. On the other hand it is possible that orchidopexy before the maturation of dark spermatogonia, which occurs at 3 - 4 months of age [71], will increase the growth of the retained testes so they become equal in size to the scrotal ones.

Nevertheless, if we undertook surgery at this early age it would lead us into a clinical dilemma since we know from our results that spontaneous descent can occur as late as six months, i.e. after the maturation process to spermatogonia has taken place.

If we assume that the spontaneously descended testes are normal, early surgery before six months of age would not be ethically acceptable. However, in this study we conclude that the growth of the spontaneously descended testes is actually impaired compared to their scrotal counterparts. As a matter of fact we have found that they grow less than testes treated at nine months.

We can only speculate on why the descended testes show impaired growth. As demonstrated in this study spontaneously descended testes are more prone to ascend to a cryptorchid position during childhood (22 %). One hypothesis about
the impaired growth is that some of the descended testes are on their “way back” to a retained position, thus being exposed to adverse conditions that in true retained testes are reflected in a lesser volume.

In support of this, other studies have indicated that testes in acquired cryptorchidism as well as retractile testes undergo adverse histological changes and also have lesser testicular volume [19, 25-27, 117]. Still, from our point of view early orchidopexy during early infancy is, despite these observations, not justifiable in unilateral cryptorchidism.

It would indeed be useful if we were able to identify endocrine markers during minipuberty in cryptorchid boys, which could predict future testicular function or spontaneous descent, and for that reason we analyzed different hormones at the age of two months. Serum inhibin B is considered to be a marker of Sertoli cell function, but studies on cryptorchid boys show contradictive results [123-125]. In our study we have not been able to demonstrate any conclusive hormone data in early infancy that could predict future spermatogenesis or spontaneous descent. The serum levels of hormones reflect the output of both testes and are therefore not easy to interpret in unilateral cryptorchidism but data were not conclusive in bilateral cases either, possibly due to an insufficient number of measurements analyzed. Consequently the predictive value of inhibin B and other hormones for the quality of spermatogenesis in adulthood remains to be established.

So before pursuing the idea of an orchidopexy age earlier than nine months we need to assess whether surgical intervention in early infancy alters the paternity potential. From studies on adults with a history of unilateral cryptorchidism it has been concluded that fertility is not compromised compared to the normal male population, whereas men with a history of bilateral cryptorchidism have a lower paternity rate [93-95, 100, 103, 107]. The men included in these studies generally had undergone surgery at a later age than recommended today, and still results
show that the late unilateral orchidopexies did not influence the paternity rate, although it ought to have increased the germ cell impairment even further.

Since men with a history of bilateral cryptorchidism have compromised fertility one might consider treatment in early infancy before the maturation of spermatogonia takes place, thereby possibly improving future spermatogenesis and paternity. This is however a highly speculative suggestion, and long-term follow-up studies are required. Nevertheless, one might consider operating bilateral cases on one side early in infancy and on the contralateral side when a spontaneous descent is unlikely to occur.

Another argument, besides improving fertility, for treating cryptorchidism is to reduce the risk of future malignancy. Cryptorchid boys are at higher risk for developing carcinoma in situ and testicular cancer as adults [46-48, 50, 59]. Orchidopexy before puberty clearly reduces the risk [56, 57], but it remains to be proven that orchidopexy before the age of one reduces the risk even further.

In morphometric and volumetric studies with five years of follow-up, we have clearly proven that orchidopexy at nine months of age is beneficial compared to surgery at the age of three; the obtained results clearly support early orchidopexy.

Before recommending surgery prior to one year of age it is important to raise the question whether surgery at this age is safe, both in regard to anaesthesia as well as to surgical complications at, or after, surgery. The Nordic consensus group has concluded that the anaesthetic risk during infancy is low and recommends that surgery should be performed at centres with pediatric anaesthesiologists. Furthermore, it is recommended that pediatric surgeons or urologists should operate on the boys if surgery is planned before the age of one year [20]. In our series we have demonstrated that the surgical method used is safe in regard to
complications at, or after, surgery and that the risk of atrophies and recurrences is low.

By showing that spontaneously descended testes often will ascend to a cryptorchid position after birth we have proved that acquired cryptorchidism exists. Other investigators have concluded that these testes undergo adverse histological changes [18, 19] and recommend treatment while others advocate a wait-and-see approach since a considerable number will descend spontaneously by puberty [9, 10, 15-17]. However, testes that did not descend required surgery and often shown to be smaller than the contralateral testis, thus indicating that they have undergone adverse changes, reflected in a lesser testicular size. Based on these data and our own findings we propose that acquired cryptorchidism should be treated upon presentation.

In summary we propose the following criteria for diagnosis and management of congenital cryptorchidism.
Fig. 20. Schematic decision tree on the management of cryptorchidism, modified from the Nordic consensus report [20].
8. Conclusions and future perspectives

The main objectives of this thesis report are to present the first prospective randomized study on the timing of treatment, to identify markers of testicular function of importance for future spermatogenesis and to investigate the growth and natural course of the spontaneously descended testes.

In both volumetric and morphology studies we have proven that surgical treatment at nine months of age is more beneficial for spermatogenesis compared to treatment at three years of age, resulting in improved testicular growth and a higher number of germ cells.

Furthermore, we have demonstrated that testicular volume correlates with the number of germ cells, thus clearly indicating that testicular volume can be used as a proxy for testicular function in congenital cryptorchidism. To our knowledge, this has never been proven in cryptorchid boys prior to this study.

However, even though testes were surgically corrected and followed up to five years they were still not showing a testicular volume equal to their scrotal counterparts. Already at birth, the retained testes had a smaller volume compared to the scrotal ones indicating that prenatal factors may play a role for testicular volume. On the other hand, we have proved that the cryptorchid position in itself has an adverse effect on testicular growth, which suggests that both pre- and postnatal factors are of importance for testicular development.

In the study we have followed boys with spontaneously descended testes and conclude that a spontaneous descent usually occurs within the first months of life and that these testes are at risk to ascend to a cryptorchid position later in childhood, thus proving that acquired cryptorchidism exists. Interestingly, at follow-up to five years, the volumetric studies show that the testicular growth is impaired compared to that of their scrotal counterparts.
(decreased ratio). The growth is also impaired to a greater extent than testes treated at the age of nine months.

We hypothesize that the descended tested are being exposed to adverse conditions by being retractile to a higher extent than scrotal testes, reflected in a lesser volume. Based on these data we have presented in the discussion a recommendation how to follow these boys clinically.

Furthermore, we wanted to test the hypothesis that hormones, analyzed early in infancy, could predict future spermatogenesis or spontaneous descents. The data collected are inconclusive, and therefore measurements of inhibin B, gonadotrophins and testosterone could neither be used as markers for future testicular function nor to predict spontaneous descent.

Results from the surgical methods used in our series show that the open inguinal surgical approach, for both palpable and non-palpable testes in infancy, is an efficient method for achieving scrotal position with low risk for atrophies or recurrences.

In summary, our findings clearly support early treatment in congenital cryptorchidism. If these findings will persist into adulthood and affect paternity still remains to be seen.

Future perspectives

Since the start of the study in 1998 we have registered more than 4 000 clinical visits, 3 300 ultrasound examinations, 2 200 blood analyses, 350 surgical interventions and 260 biopsies in more than 450 boys. The study continues with long-term follow up throughout puberty and the recruitment of newborn boys with less common phenotypes also proceeds.
In the near future we will present genetic studies on familial cryptorchidism. We have identified 80 familial cases and a number of mutations. Furthermore we will continue the morphometric studies on cryptorchid boys with testicular microcalcifications and its importance for the development of carcinoma in situ and future risk of testicular cancer.

We have presented evidence-based recommendations for the management of congenital cryptorchidism, and by following these boys further into adulthood we have a unique opportunity to gather additional data of importance for future spermatogenesis and the potential to shed some light on the controversial issues of cryptorchidism and present further recommendations how to clinically manage cryptorchid boys.
9. Acknowledgements

Since the start 12 years ago, this study has been like a long and often very inspiring journey. The work has indeed taken a long time, but its duration is also its strength, and it continues to have a great potential to shed some light on the controversial issues on cryptorchidism. Having taken part of more than 4000 clinical visits involving more than 450 boys I have now arrived at the point of writing the list of acknowledgements in my thesis book; to me this is almost as important as presenting the results themselves.

First of all, I would like to dedicate this book to all the boys and their families who have been, and still are, a part of this study. I have gotten to know many of you boys personally and some of you are now over eleven years old. When you visit us for a check-up it often feels like meeting a friend and I am really looking forward to meeting you again as this study continues. We, who stand behind this study, are proud and happy that our research has led to general recommendations for how to treat cryptorchidism. Still, without you this book would not have been published and so you have greatly contributed to the medical science. Thank you!

Agneta Nordenskjöld, supervisor
Thank you Agneta, for accepting me as a PhD student a few years ago. Besides being an excellent supervisor giving guidance through all the formalities you have enthusiastically, and with great insight, discussed everything I needed help with. It took longer than I had expected to finalize the last two manuscripts up to a point where I thought I would never be able to finish the thesis in time. But you have supported me through difficult times and encouraged me to go on a long-planned trip to South America just a few weeks before the printing of the thesis. You really believed that I could do it. Thank you Agneta! I am really looking forward to working with you on the continuation of this study and on future spin-off projects.

Martin Ritzén and Olle Söder, co-supervisors
Martin, you have been with me since the start of this study. I do not know how to thank you enough. You amaze me, not only by being a scientific wizard but also by always being there for questions and discussions. No matter if you are Africa, in between lectures, in a tennis game or riding your bike to work (often through snow) you always have a minute to discuss things. Olle, being such a dynamic person: with you in this group what could possibly go wrong?

Bengt Karpe, former supervisor
Bengt, you have contributed enormously to this thesis study as one of the initiators of the project and my guide through the first two publications - always with a good sense of humor and insight. It is really amazing that you yourself were a PhD student having Martin as a supervisor and did your thesis work on hormonal treatment of cryptorchidism. Even though you are retired now I know that you are
really pleased to see this publication and that you probably will read it cover to cover.

*Tina Granholm, former supervisor*

Tina, you have been a part of this study from day one. You were the prime initiator of this project, previously supervisor and co-author, and last but not least my roommate and friend giving insightful advice during difficult times. At the end of the day Tina: you started this.

*Co-authors and collaborators*

I consider myself very fortunate to have you involved in the work with the publications. Thank you all for being members of the group and making the study solid as a rock.

Ulf Hesser, you know of course that you have been a co-author of the first two publications. But are you also aware of having done over 3000 ultrasound examinations?! I am looking forward to working with you in new projects. Thomas Gustafsson, you have contributed enormously by setting up the infrastructure for analyzing more than 2 200 blood samples.

To the Finnish group including Erno Sundqvist, Mirja Nurmio and Jorma Toppari: without your collaboration on the morphology part we would not have been able to draw the conclusions that have led to evidence-based clinical recommendations. I learnt a lot about morphology on my trips to Tuurko -Thank you all! Jan-Bernd Stukenborg, you have contributed greatly to the morphology part, and I am already looking forward to future collaboration. I would also like to thank Anders Bergh in Umeå, for preparing a major part of the biopsies for the Finnish group.

Göran Läckgren, I would like to thank you for being one of the initiators of the project. During many years I have had interesting and rewarding discussions with you that clearly have influenced my view on cryptorchidism. Göran, thank you for contributing to this thesis work.

*Pia Kjelholm, research nurse*

Pia, without you this journey would have been more difficult. With an astonishing capability you have organized the clinical visits, blood samplings and ultrasound examinations. With great patience you have explained things to the boys and their families and sometimes persuaded them to continue to come for check-ups also after they had been treated. Thank you!

*Colleagues*

There are many of you at the clinic that I would like to thank for supporting this thesis work in different ways. Some of you have encouraged me along the way and a few of you have occasionally examined the boys when I was not available. Thank
you all for your help and support, and special thanks to you PJ for your insight and for sharing your thoughts with me when I needed it.

Lars Sundholm, mentor
Lasse, I think that you and I have forgotten that you are my external mentor in this thesis work, probably because you also are my best friend. You have encouraged and supported me from day one with personal advices through difficult times. During the last three weeks of writing you have completely bombed me with encouraging text messages and kept me laughing on the way.

Department of Pediatric Surgery
I would like to thank the Department of Pediatric Surgery for giving me the opportunity to conduct this work and finalize the thesis report.

Sylvester, Mauritz, Max-Michel and Anna
Sylvester and Mauritz, even though I have only been physically present for the last three weeks, sitting on the couch with my laptop writing the thesis, you have not once complained about me being mentally absorbed in other things than you. On the contrary you have encouraged me to continue. I have not been able to engage in your sport activities they way I usually do, so I cannot wait until I am with you on the soccer field again enjoying your progress.

To you Max-Michel I would like to say, that even though you are in London finishing your Master’s degree in journalism you have been very supportive in every possible way, constantly encouraging me but also giving me advice on the structure of the thesis. I am very proud of who you are and your values.

Anna, even though you have taken care of everything in the household, like emptying the dishwasher, doing the laundry, cooking, getting the kids to their activities and helping them with their homework, you have kept your good spirits always encouraging and supporting me. I know you have neglected doing things for yourself during these last weeks and I am looking forward to see things getting back to normal for you. Thank you Anna, you are my companion.

Grants and funding
We have gratefully received financial support from Frimurare Barnhuset, Sällskapet Barnavård and from HKH Kronprinsessan Lovisas förening för barnsjukvård.
10. References


[43] Thorup J, Cortes D, Petersen BL. The incidence of bilateral cryptorchidism is increased and the fertility potential is reduced in sons born to mothers who have smoked during pregnancy. The Journal of urology. 2006;176:734-7.


testicular texture and size in 444 men from the general population: correlation to semen


Cortes D, Thorup J, Hogdall E, Norgaard-Pedersen B, Petersen BL, Hogdall C. The


testicular texture and size in 444 men from the general population: correlation to semen

Takahara H, Cosentino MJ, Sakatoku J, Cockett AT. Significance of testicular size
measurement in andrology: II. Correlation of testicular size with testicular function. The

Noh PH, Cooper CS, Snyder HM, 3rd, Zderic SA, Canning DA, Huff DS. Testicular