Seventy percent of cryptorchid patients exhibit hypogonadotropic hypogonadism. In boys with unilateral cryptorchidism, testicular pathology caused by hormonal imbalance was bilateral; 71% of scrotal testes had a reduced number of germ cells, and 75% had impaired transformation of gonocytes into Ad (Adult dark) spermatogonia. Evidence of a relative post-pubertal gonadotropin deficiency became even more pronounced when LH plasma values were correlated with Ad spermatogonia. While both high infertility risk (HIR) and intermediate infertility risk groups had normal basal LH levels, the low infertility risk group had LH levels in the hypogonadotropic range.

Our long term, prospective follow-up study used hormonal analyses to confirm a previously observed inverse correlation between FSH and sperm count.\(^2\) Furthermore, we established that patients with bilateral cryptorchidism had higher FSH plasma values than those with unilateral cryptorchidism.\(^2\) At first glance, these findings suggest that primary testicular failure causes the hypergonadotropic hypogonadism. However, we find that gonadotropin levels are more highly correlated with the presence or absence of Ad spermatogonia than with the number of undescended testes. Patients with the greatest impairment to mini-puberty and who exhibited failed transformation of gonocytes into Ad spermatogonia had the most severe infertility.\(^2\)

The extent of testicular pathology observed in the high-risk infertility group was comparable to that observed in males with Klinefelter syndrome or idiopathic Sertoli cell only syndrome.\(^2\) However, testicular failure in XXY males or idiopathic Sertoli cell only syndrome resulted in FSH levels 3 to 5 times higher than in the controls.\(^2\) In contrast, despite identical severity of testicular pathology in cryptorchid patients with a high risk of infertility, we observed only marginal FSH elevation, indicating a relative FSH insufficiency. At least 70% of our patients had relative FSH deficiency. The finding of relative FSH and LH deficiencies in most of our patients indicates that hypogonadotropic hypogonadism is the primary etiologic factor in cryptorchidism.\(^2\) Furthermore, the normal plasma testosterone values in our cohort do not support the hypothesis of Toppari et al.\(^6\) that mild Leydig cell dysfunction and subsequent end organ dysgenesis are etiologic factors in cryptorchidism.
Cryptorchid testis: histology

The appearance of the cryptorchid testis is easily recognized, even under low magnification. It is characterized by wide, empty intercellular spaces and small seminiferous tubules with reduced numbers of germ cells. Histological sections of undescended testes show circular tubules with central spherical bodies and secondary regions with degenerated tubules.\(^7,8\)

As early as the second year of life, approximately 22% of patients with unilateral cryptorchidism are destined to lack spermatogonia in their tubules.\(^2,9\) Qualitative changes of spermatogonia can be seen as well. For example, there is an increase in the number of spermatogonia with two nuclei, and the observed spermatogonia are mostly fetal, sometimes with bizarre nuclear structures.\(^9,10\)

The marked reduction in spermatogonia in a 6-year-old cryptorchid patient includes immature forms, e.g. fetal and Ap spermatogonia. Ad spermatogonia are rarely seen and primary spermatocytes are never observed.\(^2,8-10\) Most of the observed spermatogonia show signs of incipient degeneration.\(^2,8,9\)

Mini-puberty and Ad spermatogonia

Development of male fertility depends upon successful mini-puberty\(^2,11\) and the transformation of gonocytes into Ad spermatogonia.\(^12\) During mini-puberty, which occurs between 30 and 90 days of postnatal life in male infants, a substantial increase in GnRH secretion induces an increase in gonadotropin and testosterone production.\(^13\) Testicular changes during mini-puberty are further characterized by a slight increase in testicular weight\(^14\) and volume.\(^15\) As a result, gonocytes transform into Ad spermatogonia. Ad spermatogonia have a characteristic nuclear feature that distinguishes them from other germ cells, e.g. fetal, transient, and Ap spermatogonia.\(^16\) The Ad spermatogonium is a flat cell with a long oval nucleus; this is the only form that has a region of rarefaction within the nucleus (Fig. 1).\(^16\)

Adult dark (Ad) spermatogonia appear at 3 months of age and remain for life.\(^16\) Therefore, transformation of gonocytes into Ad spermatogonia, either directly or through intermediate stages, is not simply another developmental step but a major transformation. This transformation involves the switch from the fetal reservoir of stem cells (gonocytes) to the adult reservoir of stem cells (Ad spermatogonia) from which all future germ cells are replenished. Based on results from our previous work, we know that development of Ad spermatogonia depends upon LH and T secretions.\(^7\) The expression pattern of prepubertal germ cells indicates that genes involved in meiosis and post-meiotic germ cell development are already up-regulated before puberty.\(^9\) Boys with cryptorchidism lack Ad spermatogonia and have low plasma concentrations of both basal and stimulated gonadotropin, as expected in hypogonadotropic hypogonadism.\(^18\) If transformation of gonocytes into Ad spermatogonia fails during infancy, infertility is inevitable.\(^2,10,20\) We\(^9,20\) along with others\(^21\) confirmed this finding, which was first reported in 2001.\(^19\) Kim and coworkers analyzed the histology of testicular biopsies from patients with bilateral cryptorchidism and

**Figure 1.** Histology of testicular tissue: normal and cryptorchid testes.
established the prognostic importance of Ad spermatogonia for fertility.\textsuperscript{21} Furthermore, there is strong evidence that boys with cryptorchidism will develop infertility, despite early and successful orchidopexy.\textsuperscript{2}

**EGR4 master gene for fertility in cryptorchidism**
Whole genome profiling analysis of cryptorchid testes indicates abnormalities in several developmental testicular genes. Expression of MBD2, FOXG1, TGFBRI, FDGFR1, TDRDS, CTAS, MAGEs, GAGEs, SSXs, and Spa17 were found to be lacking or under-expressed in boys in the high infertility risk group.\textsuperscript{22}

The key observation from our current work is that the early growth response gene, EGR4, was not expressed in boys in the HIR group.\textsuperscript{9} Since EGRs are pivotal for LH secretion, this provides indirect evidence that EGR 4 is important for Ad formation and that the LH-T axis is involved in this developmental process. Patients in the HIR group had severely reduced EGR4 expression, and their testicular histology showed severe tubular and Leydig cell atrophy identical to that of Egr1/Egr4 double mutant infertile mice. As in Egr mutant mice, treating boys with cryptorchidism and HIR with buserelin (a GnRH receptor agonist) normalized sperm parameters in 86% of patients who otherwise would develop infertility, despite successful orchidopexy.\textsuperscript{27} Therefore, our results suggest that EGR4 is the master gene that controls fertility development.

Although all of the patients we studied had isolated cryptorchidism in comparable undescended position and identical age, the HIR group had significantly lower EGR4 expression, indicating that descent of the epididymo-testicular unit does not require intact EGR4 function. This new observation calls into question current dogma that the undescended position itself is the cause of infertility. In this regard, we found that an RNA helicase involved in gene-specific mRNA export and protein translation during spermatogenesis was significantly down regulated in the HIR group, which coincides the decreased expression of EGR4, DDX25/GRTH. LH/HCG stimulates DDX25 via cyclic-AMP-induced androgen formation in testicular Leydig cells.\textsuperscript{27}

**Hormonal treatment and possible side effects**
One of the therapeutic options in treatment of cryptorchidism is hormonal therapy with luteinizing hormone (LH), or human chorionic gonadotropin (hCG).\textsuperscript{2} Induction of testicular descent in this manner\textsuperscript{2,28-30} improves the potential for fertility from poor to good in up to 75% of the individuals treated.\textsuperscript{31,33} There are increasing concerns about the safety of hormonal therapy.\textsuperscript{33,34} More apoptotic spermatogonia were found in patients who underwent unsuccessful hCG therapy prior to orchiopexy compared to the patients who underwent orchiopexy alone.\textsuperscript{33} However, in follow-up spermiograms there was no difference in sperm concentration, motility or percentage of normal morphology between the two groups of patients. Maturation of spermatogonia and a decrease in a number of Sertoli cells are anticipated effects of hormonal therapy, thus degeneration of Sertoli cells may be noted. In a retrospective, nonrandomized study, Cortes et al\textsuperscript{34} found fewer germ cells per tubule in 1-3 year old patients, who were treated unsuccessfully with gonadotropin releasing hormone or hCG. The number of germ cells per tubule in patients treated with orchiopexy alone was 0.14 (range = 0-0.86), while in patients previously treated with gonadotropin releasing hormone or hCG the numbers were significantly lower, 0.07 (range = 0-0.31) and 0.06 (range = 0.0025-0.21). Although these values differ statistically, they are all low enough to predict future infertility. Since this study was not randomized, there is a possibility that some patients treated with orchiopexy alone might benefit from hormonal therapy as well.

The effects of hormonal therapy on the contralateral descended testis have been studied only sporadically.\textsuperscript{35} Bergada et al.\textsuperscript{35} found stimulated maturation of germ cells in treated patients and directly related the number of mature cells to both the dose and duration of the treatment. We showed that hormonal treatment did not harm the histology of the contralateral testis but rather improved it. Patients treated with orchiopexy alone had an average of 1.33 ± 1.0 mature cells/per tubule while hormonally treated patients had 2.05 ± 1.1 germ cells per tubule (p<0.05). Hormone therapy increased the number of Ad spermatogonia per tubule as well.\textsuperscript{36}

**Hormonal treatment following orchidopexy**
Infertility induced by cryptorchidism is an endocrine disease of impaired mini-puberty. Treatment with LH-RHa following successful orchiopexy before the age of six years normalizes sperm parameters in the vast majority of patients. Normalization of the sperm counts in 86 % of males with cryptorchidism following LH-RHa treatment further refutes the hypothesis of end organ dysgenesis.\textsuperscript{27} Since 50% of patients with unilateral cryptorchidism do not belong to the high infertility risk group, they will profit from early surgery without the need for subsequent LH-RHa treatment. Testicular biopsy is the only diagnostic procedure capable of identifying patients who need to be treated with LH-RHa following successful surgery. Because of its important prognostic value, a testicular biopsy should be performed routinely during the orchiopexy.

**Treatment recommendations (age <2 years)**
1. LH-RH 1.2 mg/ day for 28 days; if no success,
2. 500 IU HCG/ week for 3 weeks; if no success,
3. orchidopexy and bilateral biopsy, if no Ad;
4. LH-RHa, 10 μg on alternate days /for 6 months.
References


