Advocating hormonal treatment to prevent adult infertility in patients diagnosed with congenital undescended testes

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Abstract

In 2007 the Nordic group came to the following unanimous conclusions: In general, hormonal treatment is not recommended, considering the poor immediate results and the possible long-term adverse effects on spermatogenesis. Thus, surgery is to be preferred. However, defective mini-puberty inducing insufficient gonadotropin secretion is one of the most common causes of nonobstructive azoospermia in men suffering from congenital isolated unilateral or bilateral cryptorchidism. The extent of alteration in the unilateral undescended testis correlate with the contralateral descended testis, indicating that unilateral cryptorchidism is a bilateral disease. Idiopathic central hypogonadism explains the phenomenon of defective mini-puberty in otherwise healthy cryptorchid boys. We therefore recommend hormonal treatment for cryptorchid boys with defective mini-puberty. Gonadotropin releasing hormone agonist (GnRHa) treatment following surgery to correct cryptorchidism restores mini-puberty via endocrinological and transcriptional effects and prevents adult infertility in most cases. Several genes are important for central hypogonadotropic hypogonadism in mammals, including many that are transcribed in both the brain and testis. At the molecular level, there is no convincing evidence that heat shock is responsible for the observed pathological testicular changes. Thus, impaired transformation of gonocytes is not the result of temperature stress but rather a hormonal imbalance. Cryptorchidism should therefore be considered a serious andrological problem that cannot be successfully treated by early orchidopexy alone.

1) The physiology of male reproduction and infertility

The major goal of treating cryptorchidism

The primary objective in treating cryptorchidism is to attain fertility. The estimated frequency of azoospermia in the general population is 0.4% (3/711) (1), with non-obstructive azoospermia occurring 25 times more frequently in unilateral and 80 times more frequently in bilateral cryptorchidism (2). Consequently, cryptorchidism stands as the most prevalent cause of azoospermia in men (3).

It is noteworthy that the incidence of azoospermia or severe oligospermia does not differ between patients who undergo surgery before and after the first year of age (p = 0.39, Fisher's Exact test; Feyles et al. 2014, as reviewed in reference (4)). Therefore, severe infertility and azoospermia manifest in cryptorchidism regardless of the age at which treatment is administered (2,5). As a result, early and ostensibly successful orchidopexy does not enhance fertility since it fails to address the underlying pathophysiological cause, namely defective mini-puberty (5).

During disrupted mini-puberty, inadequate LH secretion leads to diminished Leydig cell capacity and reduced testosterone levels, resulting in impaired Ad spermatogonia development and infertility (6). Moreover, it has been established that the differentiation of gonocytes into Ad spermatogonia is testosterone-dependent (7). Half of the patients with unilateral cryptorchidism and the majority with bilateral cryptorchidism fall into the high infertility risk (HIR) group (8). Consequently, establishing fertility is significantly reliant on a normal mini-puberty, essential for establishing a standard population of Ad spermatogonia (9,10).

In 1975, pronounced Leydig cell atrophy, commencing in early infancy, was identified as evidence supporting endocrinopathy as an etiological factor in cryptorchidism (11). However, apart from a blunted testosterone response to human chorionic gonadotropins (HCG), no evidence supports altered steroidogenesis in cryptorchid testes before puberty (12). HIR cryptorchid boys exhibit low basal and stimulated gonadotropin plasma levels comparable to those in cases of hypogonadotropic hypogonadism (13,14). Furthermore, numerous LH-RH tests have demonstrated a lower LH response to gonadotropin-releasing hormone (12). Thus, the cause of the diminished testosterone response appears to be at both the pituitary and hypothalamic levels.

Is paternity a fertility indicator?

Lee and his co-authors conducted a retrospective review of medical records using a comprehensive questionnaire, determining that unilateral cryptorchid men exhibit a normal paternity rate (15). It is essential to highlight that this study did not rely on the results of testicular biopsies. Consequently, an evaluation of testicular tissue quality and the presence of Ad spermatogonia was absent. Patients were classified as cryptorchid solely based on the fact that they had undergone surgery. Notably, the unavoidable inclusion of low, intermediate, and high infertility risk patients, along with misdiagnosed cases of retractile testes, distorts the results. Therefore, relying solely on paternity as a fertility indicator is inadequate. More significantly, only a testicular biopsy can identify patients likely to be infertile, thus benefiting from hormonal therapy. This underscores that the rationale behind testicular biopsy is both diagnostic and therapeutic.

Furthermore, the justification for testicular biopsy extends to the detection of *in situ* carcinoma, occurring in 0.4% of the cases (16).

The sperm count in long-term follow-up studies.

Approximately 47.5% of unilateral and 78% of bilateral post orchidopexy cryptorchid males exhibit sperm concentrations within the infertility range according to WHO standards (17). In the HIR group of cryptorchid men, severely decreased sperm counts were observed, with no age-related differences, indicating that a successful orchidopexy is insufficient to prevent infertility and azoospermia development (10,18).

In cases where differentiation into Ad spermatogonia had occurred (indicating functional mini-puberty), age-related differences in fertility outcomes were noted: the younger the unilateral cryptorchid boys were at surgery, the higher the adult sperm count. However, the difference in sperm count between boys younger than three years at the time of surgery (median; 156 x 10^6/ejaculate) and those older than eight years (mean; 87 x 10^6/ejaculate) is statistically significant but biologically irrelevant (10). Both groups displayed a total sperm count within the normal range.

A 20-year long-term prospective study initiated in 1985 in Philadelphia by the late John Duckett and Faruk Hadziselimovic yielded results in accordance with our previous study from 2005, emphasizing the importance of an intact hypothalamus-pituitary-testicular axis for normal fertility in cryptorchid men (17). Sperm concentrations correlated with the number of Ad spermatogonia found at the time of orchidopexy ($p < 0.001$). In the HIR group lacking Ad spermatogonia, 80% of males were oligospermic, and 20% displayed azoospermia (17). In patients with unilateral cryptorchidism, 70% of the scrotal testes showed varying degrees of impaired differentiation of Ad spermatogonia, indicating that cryptorchidism is a bilateral disease. Moreover, correlations between testicular histology and post-pubertal hormone levels confirmed various levels of gonadotropin deficiency in the majority of adult cryptorchid men. A crucial finding is that gonadotropin levels exhibit a more significant correlation with the presence or absence of Ad spermatogonia in both gonads than with unilateral or bilateral undescended testes (17).

Notably, in HIR patients, adequate treatment with low doses of GnRHa resulted in a normal sperm count in 86% of cases, and none developed azoospermia. This starkly contrasts with the results of the 'surgery-only' group, where not a single patient displayed a normal sperm count, and 20% were diagnosed with azoospermia (19).

2) RNA profiling testicular biopsies yields data that support hormonal treatment

Gene expression and function in the hypothalamus and pituitary gland

Significant differences in gene expression levels between testicular samples from high- and low-infertility risk groups were observed in various hypothalamic and pituitary genes. In 2016, we reported decreased expression of *PROK2*, *CHD7*, *FGFR1*, and *SPRY4* in HIR testis from patients with impaired LH secretion (20). Mutations in these genes are associated with Kallmann syndrome (21). Notably, EGR4, a participant in regulating luteinizing hormone secretion, is virtually not expressed in HIR samples (22). Several genes involved in pituitary development and differentiation, such as *ISL1*, *OTX2*, *PITX1*, *PITX2*, *GATA2*, *LHX2*, *LHX6*, *LHX8*, and *NHLH2*, show a lower expression signal in HIR samples compared to LIR samples. *ISL1*, a paralog of *LHX4*, gained a new function during evolution (23). *LHX4*, encoding a transcription factor controlling pituitary gland development, belongs to a large protein family with the cysteine-rich zinc-binding LIM domain.

Moreover, deletion of *OTX1* was found in six subjects with genitourinary defects, three of whom were diagnosed with cryptorchidism (24). It is worth noting that *Otx2* heterozygous male mice display compromised fertility (reduced LH levels and testicular weight) due to a defect in the development, number, and migration of GnRH neurons (25). Since *Otx1* and *Otx2* have interchangeable functions, *Otx2* could partially compensate for *Otx1* deficiency in certain patients (26).

GnRHa treatment increases the expression of genes weakly transcribed in HIR samples, such as *DLX1*, *DLX3*, *DLX6*, *EGR2*, *EGR3*, *ISL2*, *NR4A2*, *OTX1*, *OTX2*, *NHLH2*, *RUNX1*, *RUNX2*, *SIX2*, *SIX3*, *LEP*, *PCSK1*, *TAC3*, and *SOX* family members. Interestingly, lower expression signals were found in HIR samples for long noncoding RNAs (lncRNAs) participating in epigenetic processes, including AIRN, FENDRR, XIST, and HOTAIR. These data support the hypothesis that hypogonadotropic hypogonadism in boys with altered mini-puberty is the consequence of a profoundly altered gene expression program involving protein-coding genes and lncRNAs (27,28).

Gene expression and function in testis

DMRTC2, *PAX7*, *BRACHYURY/T*, and *TERT* are associated with defective mini-puberty as they exhibit decreased expression in HIR samples and respond positively to GnRHa treatment (28). Notably, *PAX7*, *EGR2*, *NRG1*, and *NRG3* appear to represent an alternative pathway activated by GnRHa, involved in regulating the differentiation of gonocytes into Ad spermatogonia. Additionally, differentially expressed genes like *EGR2*, *ETV5*, *ID4*, *TSPAN8*, and *T* are all regulated by FGF/GDNF signaling, while *FOXO1*, *KIT*, *NANOS2*, *NRG1*, *NRG3*, and *PAX7* expression is regulated by retinoic

acid (28). *PAX7, BRACHYURY/T, EGR2, NRG1*, and *NRG3* are thereby linked to both FGF/GDNF and RA signaling (28). Interestingly, four genes localized on the male-specific Y chromosome - *RBMY1B, RBMY1E, RBMY1J*, and *TSPY4* - show reduced mRNA levels in HIR samples and also positively respond to GnRHa treatment (29).

PRDM family members, such as *PRDM1*/*BLIMP* (30) and *PRDM14* (31), play crucial roles in primordial germ cell specification, differentiation, and meiotic recombination in adult germ cells (32). *PRDM9*, differentially expressed in LIR versus HIR samples, is the only PRDM member found to be downregulated in HIR samples and stimulated by GnRHa treatment (33). *PRDM9*, a downstream effector of testosterone action, is related to testosterone-regulated cell proliferation in classical testosterone target tissues. Thus, *PRDM9* is involved in establishing a normal Ad spermatogonia population, and its altered expression likely impacts male germ cell development in patients with cryptorchidism (33).

Impaired mini-puberty affects Sertoli cell development through both positive and negative regulation of morpho-regulatory and apoptotic genes. In contrast to germ cells, GnRHa treatment has a repressive effect on most Sertoli cell-specific genes, suggesting a cellular rearrangement in Sertoli cells. It is proposed that a gonadotropin-dependent increase in FASLG and GDNF expression drives Sertoli cell proliferation and germ cell self-renewal, thereby stimulating the transition of gonocytes to Ad spermatogonia. RNA-profiling experiments reveal novel testosterone-dependent genes, providing valuable insights into the transcriptional response to both defective mini-puberty and curative GnRHa treatment (34). In conclusion, *EGR4* and *PITX1*, controlled by *PROK2/CHD7/FGFR1/SPRY4*, are involved in LH deficiency, affecting germ cell transitional effectors such as *FGFR3, FGF9, NANOS2, NANOS3, SOHLH1*, and *SOHLH2*. GnRHa activates alternative pathways comprising *EGR2, EGR3, NHLH2, TAC1, TAC3, PROP1*, and *LEP*, important for LH secretion, and *DMRTC2, T, PAX7, TERT, NRG1, NRG3, RBMY1B, RBMY1E*, and *RBMY1J*, involved in the differentiation of gonocytes into Ad spermatogonia (20,28).

Temperature or transposons – what are the critical factors for fertility?

At the molecular level, there is increasing evidence that challenges the notion of heat shock being predominantly responsible for the observed pathological testicular changes in the prepubertal testis (35). No differences in the expression of heat shock protein, endoplasmic reticulum, and heat factor genes were observed between undescended and descended testes (35). Contrary to the assumption that temperature-dependent effects on cryptorchid gonads damage undescended testes before sexual maturation is complete, recent evidence aligns with the idea that germ cell loss, resulting in infertility in cryptorchidism, is a consequence of alterations in the Piwi pathway and the derepression of transposons (36).

Several members of the Tudor gene family, as well as members of the DEAD-box RNA helicase family, and *GTSF1*, *MEAL*, and *MOV10L1*, were found to exhibit significantly lower RNA signals in testicular samples from HIR patients (25,36). Patients from the low infertility risk (LIR) group consistently displayed stronger staining for GTSF1 and PIWIL4 and weaker staining for the L1 transposon compared to HIR samples (36). These findings provide initial evidence consistent with the idea that infertility in cryptorchidism is a consequence of alterations in the Piwi pathway and transposon derepression induced by the impaired function of mini-puberty.

Abnormal gametogenesis results from disturbed PIWIL biogenesis (involving four *PIWIL* genes) and insufficient *ASZ1*, *FOXA1*, and *CFTR* functions. Importantly, curative GnRHa treatment stimulates the expression of several genes involved in pituitary development and differentiation, neuronal development, and testosterone synthesis pathways (37). Thus, intact testosterone secretion and the function of P-bodies during mini-puberty contribute to the establishment of male-specific DNA methylation pathways.

Is there a role for hormonal treatment in the epididymo-testicular descent prior to surgery?

The developing gonadotropin-releasing hormone (GnRH) system is crucial for epididymo-testicular descent and is sensitive to reduced fibroblast growth factor (FGF) signaling. Our understanding of the impact of *FGFR1* in the process of epididymo-testicular descent has recently advanced. In later stages of embryonic development, the undifferentiated epididymal mesenchyme becomes a specific domain for *FGFR1* expression (38). Individuals with syndromic crypto-epididymis and those with isolated non-descent of the epididymo-testicular unit often exhibit disturbances in *FGF*, *FGFR1*, and/or genes regulating the hypothalamic-pituitary-gonadal axis (38). However, the mechanisms underlying *FGF* dysregulation may vary among different syndromes.

The primary reason for not recommending hormonal treatment for the undescended epididymo-testicular union is purportedly the low success rate, reported as only 20% (39). This statement is misleading because it does not consider the distribution of the positions of the epididymo-testicular unit before treatment. Furthermore, studies cited as evidence against hormonal treatment lack critical extended follow-up examinations. One of the initial long-term follow-up

studies demonstrated that four years after successful hormonal treatment, 65% of the testes remained descended (40). Notably, Höcht et al. published a randomized study with LH-RH or surgery groups, including 60 cryptorchid boys aged two to nine years (41,42). All patients randomized for surgery treatment alone displayed histological changes compatible with cryptorchidism, indicating that only undescended and not retractile testes were treated. LH-RH treatment was successful in 59% of the patients (42). Nine years after treatment, 52% of testes remained descended (43). Thus, LH-RH treatment is effective in achieving the permanent descent of true cryptorchid testes. Notably, the highest success was achieved when testes were localized in the pre-scrotal position (Table 1).

Author(s)	**n**	**% success**
De Muinck Keizer-Schrama *et al.* (44)	6/9	66
Borkenstein and Zobel (45)	5/9	55
Hagberg and Westphal (46)	8/17	47
Höcht (42)	3/4	75
Bica and Hadziselimovic (47)	6/11	54.5
total	28/50	56

Table 1. Descent rate of the epididymo-testicular unit from the pre-scrotal position treated with gonadotropin-releasing hormone.

3) Conclusion and recommendations

Abnormal germ cell development in cryptorchidism is not a congenital dysgenesis but rather an endocrinopathy, preceded by hormonal imbalance and perturbation of germ cell-specific gene expression during abrogated mini-puberty. GnRHa treatment of HIR (high infertility risk) patients induces a broad transcriptional response, encompassing protein-coding genes involved in pituitary development, the hypothalamic-pituitary-gonadal axis, and testosterone synthesis. Adequate treatment with low doses of GnRHa resulted in 86% of men displaying a normal sperm count, and notably, not a single patient presented with azoospermia.

Since abnormal mini-puberty is responsible for the development of infertility in cryptorchidism, post-surgical hormonal treatment is strongly recommended for the high infertility and azoospermia risk group of cryptorchid boys who underwent successful early orchidopexy. Furthermore, hormonal treatment to achieve epididymo-testicular descent as the primary choice of treatment for cryptorchidism has a long tradition in Europe. It eliminates the need for subsequent surgery, and in cases of non-responders, it facilitates orchidopexy, contributing to a reduced incidence of unilateral and the more serious bilateral complete post-surgical testicular atrophy. Therefore, the current and optimal therapeutic choice involves two steps of hormonal treatment.

A four-step treatment of prepubertal patients to prevent cryptorchidism-dependent infertility.

1. LH-RH 1.2 mg/day for 28 days.
   -no descent
2. 500 IU HCG/week for three weeks.
   -no descent
3. Orchidopexy & testicular biopsy.
   -boys with <0.05 Ad spermatogonia/tubule
4. GnRHa 10 µg intra-nasal on alternate days during six months.

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