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NEOADJUVANT GONADOTROPIN-RELEASING HORMONE THERAPY BEFORE SURGERY MAY IMPROVE THE FERTILITY INDEX IN UNDESCENDED TESTES: A PROSPECTIVE RANDOMIZED TRIAL

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ABSTRACT

Purpose: Subfertility is considered the principal consequence of cryptorchidism even after timely orchiopexy. Gonadotropin-releasing hormone (GNRH) treatment appears to improve fertility later in life by inducing germ cell maturation. In a prospectively randomized trial we investigated whether preoperative GNRH therapy improves the fertility index in primary cryptorchidism.

Materials and Methods: A total of 42 boys 11 to 100 months old (median 33.5) with 63 undescended testes were prospectively assigned to 2 groups during a 6-month period. Patients were randomized to receive either orchiopexy alone (21 patients) or with neoadjuvant GNRH therapy (21) as a nasal spray for 4 weeks at 1.2 mg per day. In both groups testicular biopsies were performed at the time of orchiopexy and histopathological fertility index was determined.

Results: Mean fertility index in the group treated with GNRH before surgery was significantly higher (1.05, SD ± 0.71) compared to the group without hormonal stimulation (0.52, SD ± 0.39, $p < 0.05$). The subgroup of neoadjuvant stimulated boys younger than 24 months achieved the best results compared to the age matched boys without hormonal treatment.

Conclusions: Neoadjuvant GNRH treatment improves fertility index in prepubertal cryptorchidism. Consequently, preoperative hormone therapy should improve fertility in adulthood. Additionally, the nasal application is well tolerated, safe and already recommended in an adjuvant setting after orchiopexy. Maximum salvage of active germinal tissue is achieved by treating cryptorchidism before the end of the first year of life.

KEY WORDS: gonadorelin; infertility, male; cryptorchidism; testis

Primary cryptorchidism occurs in 2.2% to 3.8% of full-term males. Spontaneous descent of cryptorchid testes takes place during the first year of life, resulting in an incidence of 1%.[1] A physiological testosterone and gonadotropin surge takes place at age 2 to 3 months but is absent or blunted in boys with cryptorchidism, corresponding to a form of hypogonadotropic hypogonadism.[2]

Only the normally positioned testis will guarantee unimpaired fertility. A central endocrinological dysfunction leads to failure of testis stimulation with impaired germ cell maturation, leading to subfertility.[3] The incidence of azoospermia in patients with untreated bilateral cryptorchidism is as high as 89%.[2]

Decreased fertility in adulthood is thought to be a consequence of profound alterations in testis histology that are already evident perinatally. An early histopathological finding is obvious Leydig cell hypoplasia at the end of the first month, reflecting a lack of central stimulation.[4] In contrast, total germ cell content in males younger than 6 months with cryptorchidism is within normal limits because of the presence of nontransformed gonocytes.[5,6]

Compared to normally descended testes, in which adult spermatogonia emerge from matured gonocytes, in cryptorchid testes the transformation of gonocytes is diminished. Not only does the number of spermatogonia remain severely decreased, but their development is also profoundly altered.[5,6] In a physiological state gonocytes primed by a gonadotropin surge develop into primary dark adult spermatogonia between ages 2 and 3 months.[6] If this transformation does not occur, the risk of later infertility is as high as 90%.[6] At age 4 to 6 years primary spermatocytes are observed for the first time. These 2 developmental steps are delayed, defective or both in cryptorchid testes.[7] Similar alterations, albeit to a lesser extent, are present in the contralateral descended testis.[7]

The severity of these abnormalities depends on a variety of cofactors, most importantly patient age at the time of surgery. Early surgical repositioning in the first year of life decreases the risk of subfertility, although early orchiopexy may not ensure later fertility.[8]

The most predictive parameter for fertility in adulthood is germ cell count. Testis histology correlates with postpuberty sperm counts in unilateral and bilateral cryptorchidism, and consequently not only are the sperm counts decreased, but also the paternity rates are significantly diminished.[9]

An efficient hypothalamic-pituitary-gonadal axis is necessary for testicular descent, while androgens are crucial for the inguinal-scrotal phase. Minimal endocrine dysfunction is difficult to assess because of the relative quiescence of this axis during childhood. Consequently, hormonal therapy using human chorionic gonadotropin (HCG), gonadotropin-releasing hormone (GNRH) and analogues basically leading to an increase in serum testosterone values, and, therefore, mimicking the postnatal gonadotropin surge has been widely accepted.

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Recently, hormone therapy has become more important in preventing the accompanying subfertility, most notably since normal hormone values are essential for proper spermatogenesis and timely transformation of gonocytes to adult spermatogonia.[5,6] It has been shown that postoperative treatment with the GNRH analogue buserelin in boys with a low fertility index following successful orchiopexy markedly improves fertility in adulthood.[10] Similar data were collected for the GNRH analogue nafarelin, where it has been demonstrated that the fertility index improved in response to the therapy.[11] Promising results were also achieved before orchiopexy when GNRH and HCG were mainly used in combination or consecutively.[8]

The purpose of this study was to investigate whether preoperative administration of GNRH affects the fertility index in primary undescended inguinal testes in a prospective randomized fashion. This topic seems particularly important, since it is evident that infertility occurs despite accurately timed surgery for cryptorchidism in childhood.[2,3,6]

MATERIALS AND METHODS

A total of 42 boys with a median age of 33.5 months (range 11 to 100) with unilateral (21 patients, median age 34 months, range 11 to 100) or bilateral (21 patients, median age 34 months, range 13 to 100) undescended testes were prospectively randomized to 2 treatment arms after informed consent. In total 63 testes were investigated. Exclusion criteria consisted of retractile testis, previous groin surgery, ectopia, secondary cryptorchidism, preterm birth, hormonal disorders and hormonal pretreatment. One study group received 1.2 mg intranasal gonadorelin daily for 4 weeks before orchiopexy (30 testes, median patient age 32 months, range 11 to 100), and the other (33 testes, median patient age 47 months, range 13 to 100) underwent surgery alone. The age difference of the 2 groups was not statistically significant ($p = 0.15$), guaranteeing comparability.

Orchiopexy was performed 4 weeks after completed hormonal therapy. Testicular biopsies of all undescended testes were taken at the time of surgery. Biopsies were fixed in Bouin's solution and embedded in paraffin. Routine 4 μm sections were stained with hematoxylin and eosin. Histological sections were analyzed at $\times 300$ magnification labeled with a numerical code and evaluated while blinded to clinical data. Fertility indices were calculated by counting the number of adult spermatogonia per tubule in at least 80 tubules according to the method described by McAleer et al.[12] Comparisons of the fertility index of pretreated and nonpretreated patients for statistical evaluation according to age and presence of unilateral or bilateral cryptorchidism were performed. To stratify the 2 groups, we defined 3 age groups—0 to 24 months (16 testes), 25 to 72 months (31) and 73 months or older (16).

Differences in the fertility index between the defined groups were analyzed by the Mann-Whitney U test. Data are expressed as mean plus or minus standard deviation (SD), or mean and range with statistical significance considered at $p < 0.05$. SPSS for Windows 11.5 software (SPSS, Inc., Chicago, Illinois) was used for all analysis.[9]

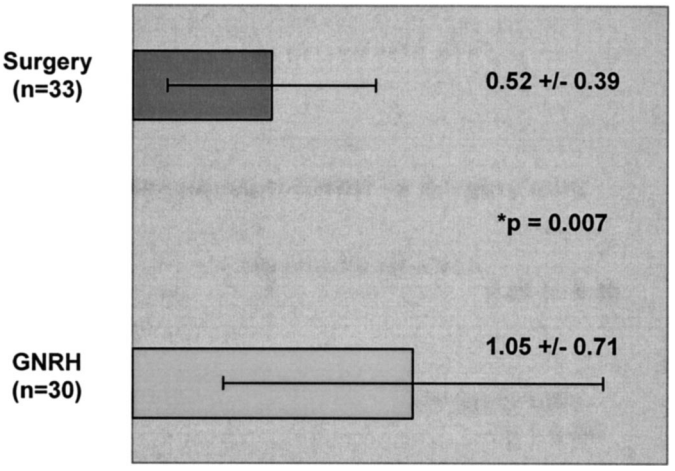

FIG. 1. Average amount of spermatogonia per tubule plus or minus SD with and without GNRH treatment in all investigated age groups. *n*, number of testes. Asterisk indicates statistical significance.

RESULTS

Mean fertility index in children treated with gonadorelin was 1.05 (SD ± 0.71, range 0.27 to 3.33) spermatogonia per tubule. Patients who underwent surgery only had significantly fewer ($p = 0.007$) spermatogonia per tubule (mean 0.52, SD ± 0.39, range 0 to 1.17). Testis specimens of patients receiving preoperative gonadorelin treatment comprised 101.9% more spermatogonia per tubule compared to those of patients receiving surgery only (fig. 1). The highest fertility index (3.33) was seen in an 11-month-old boy after hormonal treatment. The lowest value in this group was 0.27 in a 33-month-old boy. In 2 cases a Sertoli cell only state was observed. Both boys (ages 28 and 68 months) underwent surgery only. The highest recorded value in the surgery only group was 1.17 (table 1).

In the subgroup of patients younger than 24 months the mean quantity of spermatogonia per tubule was 1.27 (SD ± 0.98, range 0.39 to 3.33) in the GNRH group and 0.29 (SD ± 0.25, range 0.01 to 0.52) in the orchiopexy only group (fig. 2). Boys 25 to 72 months old after GNRH treatment displayed values of 0.94 (SD ± 0.54, range 0.27 to 2) with GNRH and 0.56 (SD ± 0.42, range 0 to 1.12) without GNRH treatment. After age 73 months neoadjuvant administration of GNRH results in a fertility index of 0.83 (SD ± 0.22, range 0.61 to 1.04) compared to 0.57 (SD ± 0.40, range 0.2 to 1.17) for surgery only. Statistical significance could only be observed in boys treated before age 2 years ($p = 0.03$). After age 2 years no statistically significant difference was found (25 to 72 months $p = 0.12$, and after 73 months $p = 0.26$), although a positive trend may be assumed.

Furthermore, the influence of laterality on fertility index was analyzed (table 2). The highest mean value of 1.11 (SD ± 0.84, range 0.27 to 3.33) was achieved in unilateral inguinal testes treated with GNRH vs 0.47 (SD ± 0.39, range 0.01 to 1.17) in the surgery only group ($p = 0.03$). Nevertheless,

TABLE 1. *Patient characteristics*

	Gonadotropin-Releasing Hormone + Surgery Group	Surgery Only Group
No. pts (No. testes)	21 (30)	21 (33)
Unilat cryptorchidism	12	9
Bilat cryptorchidism	9	12
Median mos age at orchiopexy (range)	32 (11–100)	47 (13–100)
Mean spermatogonia/tubule ± SD (range)	1.05 ± 0.71 (0.27–3.33)	0.52 ± 0.39 (0–1.17)

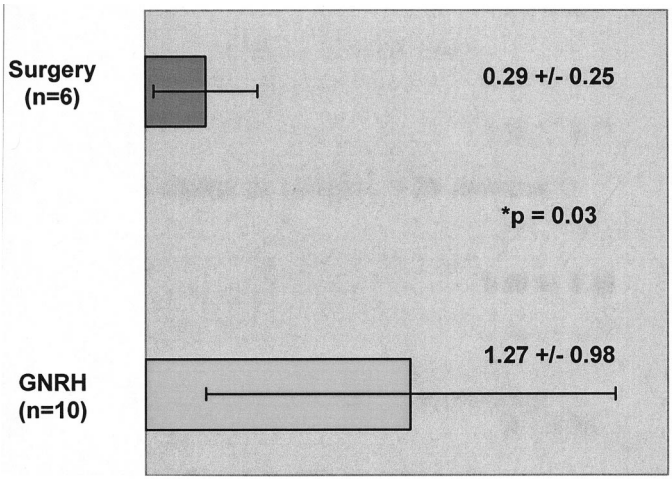

FIG. 2. Statistical analysis demonstrates average spermatogonia per tubule ratio plus or minus SD in both groups younger than 24 months. *n*, number of testes. Asterisk indicates statistical significance.

conclusions are limited due to the significant age difference between the groups (median age 31 months in GNRH group, 73 months in surgery only group, $p = 0.005$).

In bilateral cryptorchidism the mean fertility index was significantly higher after hormonal treatment. A mean fertility index of 0.96 (SD ± 0.47, range 0.5 to 2) was observed using GNRH, whereas it was 0.56 (SD ± 0.38, range 0 to 1.12) without hormonal stimulation ($p = 0.005$, fig. 3). These 2 groups are statistically comparable (median age 33 months in GNRH group vs 34 months in surgery only group, $p = 0.34$). Furthermore, a decrease in total Leydig cell population was noted in all cases. This diminution was strongest in cases with a low fertility index. No recurrence of cryptorchidism was observed in either group, and no side effects were noted in the group with hormonal pretreatment.

DISCUSSION

Although surgical orchiopexy remains the gold standard for managing cryptorchidism, it is obvious that hormone therapy positively affects testis histology and fertility potential.[10,11,13] Previous studies suggest that the 2 fundamental androgen dependent steps in the maturation of germ cells that occur in the prepubertal testis are defective in boys with cryptorchidism.[5] Based on the fact that a normal hypothalamic-pituitary-gonadal axis is a precondition for testis development, studies investigating the effects of hormones and hormone analogues on cryptorchidism and fertility in a neoadjuvant or adjuvant setting were performed.

Despite excellent research on testis histology after hormonal treatment, there is still no consensus concerning the appropriate therapeutic regimen. It has been found that native GNRH nasal solution was ineffective at inducing testicular descent as early as 1986.[14] Subsequently, longer lasting analogues such as buserelin have been investigated in open and placebo controlled, double-blind studies.[15,16] In 1 study it was concluded that buserelin induces testicular descent (17%) and increases germ cell count.[16] Testis biopsies were performed in boys who did not respond to the therapy in terms of testicular descent. The spermatogonia per tubule ratio was significantly improved after buserelin treatment.

GNRH analogues have also been administered after successful orchiopexy.[10] After a testis biopsy at operation infertility risk groups were determined (less than 0.2 germ cells per tubule) and then treated with buserelin during a 6-month period. Patients reevaluated after puberty by sperm cell counts displayed higher sperm cell counts after adjuvant GNRH treatment. Nevertheless, the number of patients in this study was small, and moreover they received pretreatment with HCG before orchiopexy. Huff et al published similar results for the GNRH analogue nafarelin.[11] Positive effects of GNRH analogues were also observed in animal experiments.

To prevent loss of the fertility potential, timely orchiopexy as well as combined preoperative and postoperative hormone therapy with GNRH and HCG is currently recommended.[8] However, one should be cautious in applying HCG, which causes inflammation-like changes in the human testes, as well as an increase in germ cell apoptosis with smaller testis volumes and higher follicle-stimulating hormone (FSH) levels in adulthood.[17,18] Cortes et al further evaluated the safety of hormonal treatment in cryptorchidism in 1 to 3-year-old boys.[19] They compared 19 boys who had undergone GNRH pretreatment with 45 patients without preconditioning. The average fertility index after surgery only was 0.14 vs 0.07 after pretreatment. Both values are strikingly lower than expected values in healthy individuals.[12] Furthermore, 20% of the boys in the GNRH group had a Sertoli cell only syndrome. Proliferation of germ cells is not likely in such patients, since stem cells are absent. The high percentage of Sertoli cell only conditions might limit the conclusions. In contrast to this observation, Cortes et al proposed additional hormonal therapy after successful orchiopexy because of a relative deficiency of FSH.[3]

Since morphological alterations of spermatogenesis already start by the end of the first year of life, surgical correction is recommended before that age.[4,12] However, timely orchiopexy is only one part of successful fertility preservation in this particular population, since surgery alone does not replace the lacking gonadotropin surge.[3,6] To achieve higher fertility rates, surgery should be accompanied by GNRH treatment, which provides sufficient transformation of all germ cell precursors, creating a greater stem cell pool for postpubertal spermatogenesis.

Preoperative application of GNRH nasal spray significantly improves the fertility index in primary cryptorchidism, with a significantly positive impact on fertility in adulthood.[9] In our study we focused above all on adult spermatogonia incidence to predict future fertility capability.

TABLE 2. *Unilateral versus bilateral cases*

	Gonadotropin-Releasing Hormone + Surgery Group	Surgery Only Group
Cryptorchidism:		
Unilat	12	9
Bilat	9 (18 testes)	12 (24 testes)
Median mos age (range):		
Unilat*	31 (11–85)	73 (13–100)
Bilat†	33 (13–100)	34 (13–63)
Mean spermatogonia/tubule ± SD (range):		
Unilat‡	1.11 ± 0.84 (0.27–3.33)	0.47 ± 0.39 (0.01–1.17)
Bilat*	0.96 ± 0.47 (0.5–2)	0.56 ± 0.38 (0–1.12)

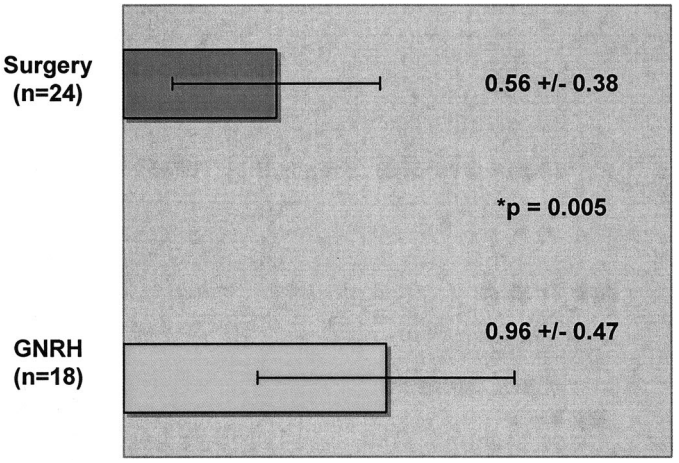

FIG. 3. Histological fertility index plus or minus SD with and without GNRH treatment in bilateral cryptorchidism. Asterisk indicates statistical significance.

Improvement could be seen in all investigated age groups up to 100 months. Nevertheless, the highest fertility indices could be achieved before age 2 years. In this age group the difference compared to patients without hormonal pretreatment was significant.

Overall fertility indices in the GNRH group are negatively correlated with patient age. This finding corresponds to previous data by other authors who found significantly diminished spermatogonia per tubule indices already at the end of the first year of life compared to expected values in a healthy population.[4,5,12] Nevertheless, preoperative GNRH application even in older boys improves fertility indices as revealed in our study. Although this outcome is not statistically significant, one may assume a positive trend. A lack of significance may partly be explained by the relatively small groups. Mean fertility index in boys older than 73 months being treated with gonadorelin was 0.83, whereas it was 0.33 in a group of patients receiving surgery only. Still, even after hormonal pretreatment mean fertility indices are substantially decreased compared to healthy controls, in whom 4.3 spermatogonia per tubule were found.[12] Between ages 2 and 6 years the mean fertility index after GNRH pretreatment was 0.94 vs 0.36 in the surgery only group. Before age 2 years GNRH pretreatment increases the fertility index to 1.27, which corresponds to expected values of 1.7 in healthy individuals.[12]

In cases of bilateral cryptorchidism fertility indices are significantly higher after hormone therapy, which may reflect a lack of central stimulation in the etiology of subfertility.[4] This finding is particularly interesting since there is further evidence for a relative deficiency of FSH.[3] Between ages 12 and 36 months 85% to 96.3% of all boys with cryptorchidism are supposed to have a pathological spermatogonia per tubule ratio, which further advocates additional preoperative hormonal treatment.[6,20]

CONCLUSIONS

We have shown that preoperative treatment with GNRH significantly improves the fertility index in primary cryptorchid testes. Nasal application is appropriate for children, without side effects. The most advantageous fertility prognosis is achieved with neoadjuvant GNRH administration and orchiopexy within the first year of life, whereby normal spermatogonia per tubule indices can be achieved. In older boys preoperative GNRH therapy is as beneficial, although normal testis histology is not restored. Based on our data, we propose preoperative GNRH therapy in every child with cryptorchidism for maximum transformation of gonocytes to adult dark spermatogonia, to promote the first postnatal maturational step of germ cells. The average fertility index is increased above the prognostically important threshold of 0.6, accordingly improving the individual fertility potential.

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