A diminished postnatal surge of Ad spermatogonia in cryptorchid infants is additional evidence for hypogonadotropic hypogonadism

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Summary

Background: The aim of this study was to describe the development of Ad spermatogonia in both unilateral and bilateral cryptorchid infants compared to a control population of comparable age and to note particularly the fate of Ad spermatogonia during the normal surge of testosterone and gonadotropin.

Methods: The incidence and development of Ad (dark) adult type of spermatogonia were assessed in 270 testicular biopsies from 159 cryptorchid infants at 1–12 months of age. These results were compared to the control population of the same age.

Results: The number of Ad spermatogonia increased markedly after five months of life in the control population. The scrotal testes of unilateral cryptorchid infants also had an increase in the number of Ad spermatogonia but it was distinctly lower than that of the control population. In contrast, this surge was completely absent in the cryptorchid testes. The number of Ad spermatogonia in unilateral cryptorchid testes correlated in a nonlinear fashion with those in the contralateral scrotal testes. The total number of germ cells in the cryptorchid testes in the first six months of life is normal, after which time it declines rapidly.

Conclusion: The impaired transformation of germ cells into Ad spermatogonia in both unilateral and bilateral cryptorchid infant testes during mini-puberty underscores the importance of hypogonadotropic-hypogonadism in the pathogenesis of this disease.

Key words: cryptorchidism; Ad spermatogonia; treatment; fertility

Introduction

Thirty-five percent of cryptorchid boys who undergo orchidopexy before six months of age will be infertile even though they have a normal total number of germ cells at the time of surgery [1, 2]. Their infertility is caused by defective transformation of germ cells into Ad (dark) spermatogonia, the progenitors of spermatozoa [1, 2]. A temporary surge of gonadotropins and testosterone occurs in boys at between 2–6 months of age [3–5]. This surge of plasma testosterone in cryptorchid infants is lower than in the age matched control population [3–7], although some investigators have found the level of testosterone to be normal [8]. The aim of this study was to describe the development of Ad spermatogonia in both unilateral and bilateral cryptorchid infants compared to a control population of comparable age and in particular to record the fate of Ad spermatogonia during the surges of testosterone and gonadotropin.

Patients and methods

Between 1971 and 2003 our institutions received 270 testicular biopsies for histological examination from 159 cryptorchid infants aged 1–12 months. Biopsies from 125 unilateral cryptorchid testes, 111 from their contralateral scrotal testes, together with 34 bilateral cryptorchid testes were compared to 50 biopsies of controls of comparable ages (Table 1). The testicular tissue was fixed in 2% glutaraldehyde and embedded in Epon. Semi-thin sections were stained with toluidine-blue and analysed with light microscopy. Ad spermatogonia were assessed in seminif-
erous tubular cross sections, and at least 50 tubular cross sections per biopsy were analysed by one single investigator, namely the corresponding author.

**Ethical considerations:** In accordance with the Helsinki declaration, the Institutional Review Board (IRB) as well as the Independent Ethics Committee (IEC) of the University Children’s Hospital, Basel and the Kindertagesklinik, Liestal, approved all aspects of this study. In particular, approval was given for research involving the use of material (data, documents, records, or specimens) that has been collected, or will be collected solely for non-research purposes (such as medical treatment or diagnosis).

**Statistical analyses:** Differences between two independent data sets were assessed using the Mann-Whitney U test. For related samples (i.e., scrotal versus undescended testes in unilateral cryptorchidism), the Wilcoxon matched-pairs signed-ranks test was used. Correlation was determined by the Spearman rank correlation coefficient. Non-parametric linear regression has been computed according to Passing and Bablok [9, 10]. Non-linearity was checked by a one sample runs test. Statistical significance level was 0.05% (5%).

### Results

During the first five months of life, the total number of germ cells was within the normal range in both the undescended testes and the contralateral scrotal testes. None of the 270 testes had a Sertoli cell-only syndrome. No severe testicular lesions, particularly focal types, were observed in any of the unilateral and bilateral cryptorchid testes. The most prominent pathological changes were found in the interstitium. The Leydig cells were atrophic in all cryptorchid testes as early as the first month of life. Very few Ad spermatogonia developed during the first five months. The undescended testes of unilateral cryptorchid boys had fewer Ad spermatogonia ($\bar{x}: 0.01$) compared to the scrotal partners ($\bar{x}: 0.03$) [$p <0.05$] and to the control population ($\bar{x}: 0.03$) [$p <0.05$]. No statistical difference between the contralateral scrotal testes ($\bar{x}: 0.03$) and the control testes ($\bar{x}: 0.03$) was observed (figure 1). The number of Ad spermatogonia in the tubules of infant testes increased sharply after five months ($p <0.01$) (figure 1). Transformation from progenitor cells, gonocytes and foetal spermatogonia into Ad spermatogonia occurred more often in the control population ($\bar{x}: 0.2$) than in the contralateral scrotal testes ($\bar{x}: 0.08$) [$p <0.01$], and it was completely lacking in the undescended testes ($\bar{x}: 0.00$) (figure 1). Thus, during the first twelve months of life, Ad spermatogonia in unilateral cryptorchid testes were fewer ($\bar{x}: 0.01$) and differed from the number of those in both the scrotal ($0.08$) and the control testes ($\bar{x}: 0.1$) [$p <0.01$] (figure 1). The number of Ad spermatogonia in infants older than 6 months remained constant, irrespective of the position of the testes, whether cryptorchid ($\bar{x}: 0.01$), scrotal ($\bar{x}: 0.09$), or normal ($\bar{x}: 0.16$) (figure 1). There was a non-linear correlation of the number of Ad spermatogonia in the contralateral scrotal testes and the unilateral cryptorchid testes ($p <0.0001$) (figure 2). If the number of Ad spermatogonia in the scrotal testes was <0.05 per tubular cross section, the majority of cryptorchid testes were devoid of Ad spermatogonia (figure 2). The testes from bilateral cryptorchid boys older than 8 months had fewer Ad spermatogonia ($\bar{x}: 0.00$) compared to those with unilateral cryptorchidism ($\bar{x}: 0.01$) [$p <0.001$] (figure 1).
An important maturational step in the hypothalamic-pituitary-testicular axis in boys occurs postnatally. The serum concentrations of LH are within the adolescent range by 1–2 weeks after birth and peak between 1–2 months, declining slowly thereafter to typical prepubertal childhood levels usually by 4–6 months of age [3–5, 11–14]. In response to the increased gonadotropins, there is a rapid increase in testosterone and its bioactive form [3–5, 11–15]. Concentrations of testosterone subsequently decrease analogously with LH [12, 13]. Accompanying, and probably stimulated by the increased secretion of gonadotropin and testosterone, germ cells proliferate during the first 100 postnatal days [16]. Additionally, testosterone induces the transformation of gonocytes into spermatogonia [17].

The postnatal surge of LH, testosterone and its bioactive form is absent or diminished in hypopituitary infants [18, 19], cryptorchid infants [5, 6, 13] and those with complete androgen insensitivity syndrome [11]. These hormonal disparities are accompanied by cytological abnormalities in the testes of cryptorchid infants, including reductions in the number of Leydig cells and the maturational process of the transformation of gonocytes into Ad spermatogonia [2, 17]. Patients with complete androgen insensitivity syndrome also have defective transformation of Ad spermatogonia [20]. An important discovery in our study is the fact that the contralateral descended testis in unilateral cryptorchid boys is also abnormal, indicating that the abnormality is an endocrinopathy. Leydig cell atrophy has been shown to be an early pathological finding in cryptorchid testes [21]. This atrophy is caused by insufficient gonadotropin stimulation [21]. The finding that the contralateral scrotal testis in unilateral cryptorchidism is also abnormal indicates that both uni- and bilateral cryptorchidism endocrinopathy is the cause of this disease. In unilateral cryptorchidism, however, this endocrinopathy is less severely expressed. The LH-RH treatment alleviates atrophy of the Leydig cells and stimulates their precursor cells to mature [21].

We have established for the first time that the development and increase in the number of Ad spermatogonia parallels the surges of gonadotropins and testosterone and that the defective development of Ad spermatogonia occurs in both the cryptorchid testes and the scrotal testes, indicating an insufficient testosterone priming effect. This handicap remains throughout the entire first year of life. Furthermore, cryptorchid boys lacking germ cells experienced the most severe form of hypogonadotropic hypogonadism [22]. Thus, the postnatal increase of testosterone is not an adaptational phenomenon [23] but a pivotal step in the development of the adult pool of stem cells from which all future germ cells are replenished. An additional hypothesis suggested to explain the inability of gonocytes to transform into Ad spermatogonia may be a congenital disturbance of germ cell division. However, the lack of Ad spermatogonia in patients with complete androgen insensitivity syndrome corroborates the first hypothesis, namely that testosterone is a requisite for the transformation of gonocytes into Ad spermatogonia.

Discussion

An important maturational step in the hypothalamic-pituitary-testicular axis in boys occurs postnatally. The serum concentrations of LH are within the adolescent range by 1–2 weeks after birth and peak between 1–2 months, declining slowly thereafter to typical prepubertal childhood levels usually by 4–6 months of age [3–5, 11–14]. In response to the increased gonadotropins, there is a rapid increase in testosterone and its bioactive form [3–5, 11–15]. Concentrations of testosterone subsequently decrease analogously with LH [12, 13]. Accompanying, and probably stimulated by the increased secretion of gonadotropin and testosterone, germ cells proliferate during the first 100 postnatal days [16]. Additionally, testosterone induces the transformation of gonocytes into spermatogonia [17].

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Conclusion

“Mini-puberty”, the primal postnatal hormonal surge, transforms germ cells into Ad spermatogonia and thus establishes the fundamentals of male fertility. Despite a successful orchidopexy, fertility is guaranteed later in life only when Ad spermatogonia are present at the time of surgery [1, 2].

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