Peer reviewed article

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

Faruk Hadziselimovic[a], *Lyman R. Emmons*[a], *Mauro W. Buser*[b]

[a] Kindertagesklinik, Liestal, Switzerland
[b] Statistik, Riehen, Switzerland

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 non-linear 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

This study was supported in part by a Swiss National Foundation grant.

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 ($\tilde{x}$: 0.01) compared to the scrotal partners ($\tilde{x}$: 0.03) [$p < 0.05$] and to the control population ($\tilde{x}$: 0.03) [$p < 0.05$]. No statistical difference between the contralateral scrotal testes ($\tilde{x}$: 0.03) and the control testes ($\tilde{x}$: 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 ($\tilde{x}$: 0.2) than in the contralateral scrotal testes ($\tilde{x}$: 0.08) [$p < 0.01$], and it was completely lacking in the undescended testes ($\tilde{x}$: 0.00) (figure 1). Thus, during the first twelve months of life, Ad spermatogonia in unilateral cryptorchid testes were fewer ($\tilde{x}$: 0.01) and differed from the number of those in both the scrotal (0.08) and the control testes ($\tilde{x}$: 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 ($\tilde{x}$: 0.01), scrotal ($\tilde{x}$: 0.09), or normal ($\tilde{x}$: 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 ($\tilde{x}$: 0.00) compared to those with unilateral cryptorchidism ($\tilde{x}$: 0.01) [$p < 0.001$] (figure 1).

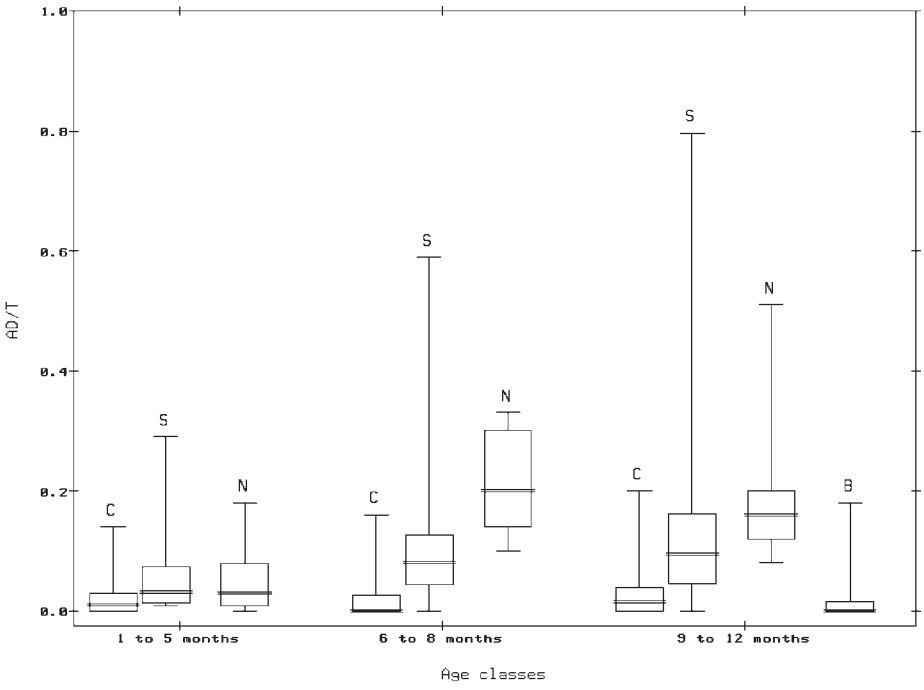


Figure 1

Incidence of Ad spermatogonia in three age groups of cryptorchid infants and control population. C: unilateral cryptorchid testis, S: its scrotal partner, N: normal control testis, B: bilateral cryptorchid testes, 9–12 months of age. Inter quartile range (box) Q_3–Q_1 (i.e., the central most 50% of data), Median: line within the bar, and first and third quartiles are presented.

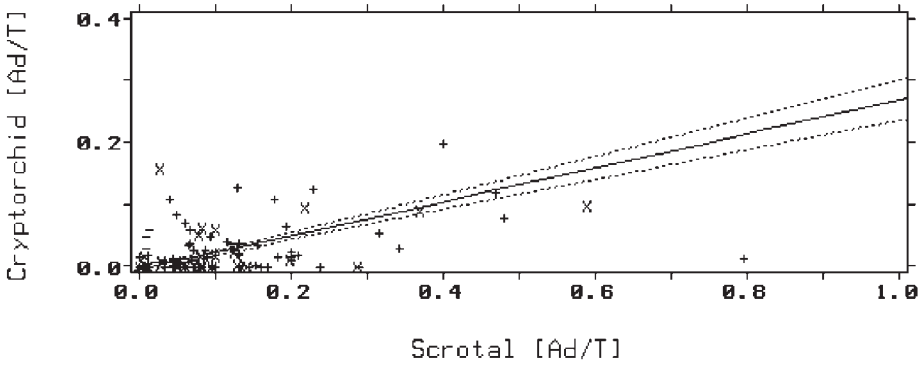


Figure 2

Correlation between the number of Ad spermatogonia per tubular cross section in unilateral cryptorchid infants and their scrotal partners (–) 0–5 months, (x) 6–8 months, (+) 9–12 months. Line: Passing-Bablok regression line: dotted line: auxiliary 95% confidence belt.

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].

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.

Conclusion

"Mini-puberty", the primal postnatal hormonal surge, transforms germ cells into Ad spermatogonia and thus establishes the fundaments 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].

Correspondence:
Professor Dr. med. Faruk Hadziselimovic
Kindertagesklinik
Oristalstrasse 87a
CH-4410 Liestal
E-Mail: ktk@kindertagesklinik.ch

References

1 Hadziselimovic F, Herzog B. Importance of early postnatal germ cell maturation for fertility of cryptorchid males. Horm Res 2001;55:6–10.

2 Hadziselimovic F, Herzog B. The importance of both an early orchidopexy and germ cell maturation for fertility in cryptorchidism. Lancet 2001;358:1156–7.

3 Job JC, Toublanc JE, Chaussain JL, Gendrel D, Garnier P, Roger M. Endocrine and immunological findings in cryptorchid infants. Horm Res 1988;30:167–72.

4 Facchinetti F, Barcci R, Sardelli S, Vanni MG, Bagnoli F, Genazzani AR. Possible testicular 3 beta-hydroxysteroid dehydrogenase deficiency in cryptorchid neonates Arch Androl 1983; 3:253–9.

5 Raivio T, Toppari J, Keleva M, Virtanen H, Haavisto A-M, Dunkel L, et al. Serum androgen bioactivity in cryptorchid and non-cryptorchid boys during the postnatal reproductive hormone surge. J Clin Endocrinol Metab 2003;88:2597–9.

6 Hamza AF, Elrahim M, Elnagar O, Maaty SA, Bassiouny E, Jehannin B. Testicular descent: When to interfere? Eur J Pediatr Surg 2001;11:173–6.

7 Baker BA, Morley R, Lucas A. Plasma testosterone in preterm infants with cryptorchidism. Arch Dis Child 1988; 63:1198.

8 DeMuinck Keizer-Schrama SMPF. Hormonal aspects of cryptorchidism. In: de Muinck Keizer-Schrama SMPF, Hazebroek FWJ, eds. Treatment of cryptorchidism. Why-How-When. Alblasserdam: Davids, 1986:83–140.

9 Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. J Clin Chem Clin Biochem 1983;21:709–20.

10. Bablok W, Passing H, Bender R, Schneider B. A general regression procedure for method transformation. J Clin Chem Clin Biochem 1988; 26:783–90.

11 Bouvattier C, Carel J-C, Lecointre C, David A, Sultan C, Bertrand A-M, et al. Postnatal changes of T, LH, and FSH in 46 XY infants with mutations in the AR gene. J Clin Endocrinol Metab 2002;87:29–32.

12 Hammond GL, Koivisto M, Kouvalainen K, Vihko R. Serum steroids and pituitary hormones in infants with particular reference to testicular activity. J Clin Endorinol Metab 1979; 49: 40–5.

13 Gendrel D, Chaussain JL, Roger M, Job JC. Simultaneous postnatal rise of plasma LH and testosterone in male infants. J Pediatr 1980;97:600–2.

14 Burger HG, Yamada Y, Bangah ML, McCloud PI, Warne GL. Serum gonadotropin, sex steroid, and immunoactive inhibin levels in the first two years of life. J Clin Endocrinol Metab 1991;72:682–6.

15 Winter JS, Hughes IA, Reyes FI, Fairman C. Pituitary-gonadal relations in infancy: 2 Patterns of serum gonadal steroid concentrations in man from birth to two years of age. J Clin Endocrinol Metab 1976;42:679–86.

16 Muller J, Skakkeback NE. Fluctuations in the number of germ cells during the late foetal and early postnatal periods in boys. Acta Endocrinol (Copenh) 1964;105:271–4.

17 Hadziselimovic F, Thommen L, Girard J. Herzog B. The significance of postnatal gonadotropin surge for testicular development in normal and cryptorchid testes. J Urol 1986;136: 274–6.

18 Job JC, Toublanc JE, Chaussain JL, Gendrel D, Roger M, Canlorbe P. The pituitary-gonadal axis in cryptorchid infants and children. Eur J Pediatr 1987;146(Suppl 2):2–5.

19 Main KM, Schmidt IM, Skakkeback NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. J Clin Endocrinol Metab 2000;65:4905–7.

20 Hadziselimovic F, Huff D. Gonadal differentiation – normal and abnormal testicular development. Adv Exp Med Biol 2002; 511:15–21.

21 Hadziselimovic F, Herzog B, Girard J, Stalder G. Cryptorchidism-Histology, Fertility and Treatment. Prog reprod Biol Med 1984;10:1–15.

22 Hadziselimovic F, Girard J, Herzog B. Lack of germ cells and endocrinology in cryptorchid boys from one to six years of life. In: Bierich J, Giarola A, eds. Cryptorchidism. London: Academic Press, 1979:129–34.

23 Huhtaniemi I. Gonadotrophin actions on the testis – Genotypes and phenotypes of gonadotrophin and gonadotrophin receptor mutations. In: Söder O, ed. The developing testis. Physiology and Pathophysiology. Basel, Karger: Endocr Dev 2003:5: 81–103.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

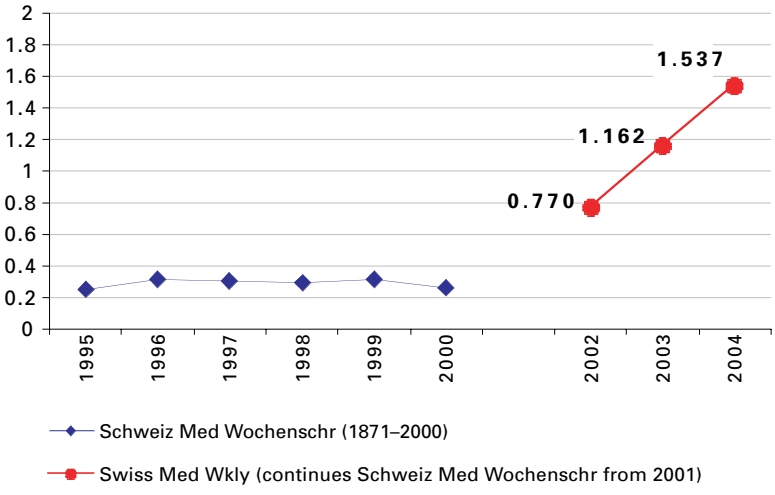
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch