European Journal of Endocrinology (2007) **157** 345–350 ISSN 0804-4643

CLINICAL STUDY

High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome

Lise Aksglaede[1], Jørgen H Petersen[1,2], Katharina M Main[1], Niels E Skakkebæk[1] and Anders Juul[1]

[1]*University Department of Growth and Reproduction GR, Rigshospitalet, University of Copenhagen, Section 5064, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark and* [2]*Department of Biostatistics, University of Copenhagen, Copenhagen Ø, Denmark*

(Correspondence should be addressed to L Aksglaede; Email: lise.aksglaede@rh.regionh.dk)

Abstract

Objective: Klinefelter's syndrome (KS) is associated with hypergonadotrophic hypogonadism in adulthood. However, limited information exists about the age at which hypogonadism occurs. The hypothalamic–pituitary–gonadal (HPG) axis is transiently activated during the first months of life, offering the opportunity to study testicular function by spontaneous, basal hormone levels. The aim of this study was to evaluate the HPG axis in KS infants.

Design: Cross-sectional study.

Methods: Ten KS infants aged 3.1 months (range 1.8–3.8) and 613 healthy controls aged 3.0 months (range 2.0–4.5). Serum levels of total and free testosterone (T), LH, FSH, inhibin B and sex hormone-binding globulin (SHBG) were determined.

Results: KS infants had significantly higher concentrations of total T (5.0 (2.2–11.2) vs 3.4 (0.7–8.3) nmol/l, $P=0.02$), free T (31.6 (18.2–61.8) vs 22.1 (4.3–48.4) pmol/l, $P=0.01$), LH (3.3 (1.3–4.6) vs 1.7 (0.6–4.3) IU/l, $P=0.005$) and FSH (1.7 (1.1–4.1) vs 1.2 (0.4–3.0) IU/l, $P=0.007$) than controls. SHBG and inhibin B did not differ from controls. LH/T and LH/free T ratios were normal, whereas the FSH/inhibin B ratio was elevated (6.5 (2.7–16.9) vs 3.0 (0.78–11.4), $P=0.005$) when compared to controls. The majority of KS infants had normal bivariate hormonal combinations.

Conclusion: We found increased FSH/inhibin B ratio as a possible sign of Sertoli cell dysfunction. However, serum levels of T were high normal suggesting an altered pituitary–gonadal set point.

European Journal of Endocrinology **157** 345–350

Introduction

Klinefelter's syndrome (KS) is caused by a sex chromosome aberration (most commonly 47,XXY) resulting in hypergonadotrophic hypogonadism in adulthood due to primary testicular failure. Boys with KS usually enter puberty at the expected time with increasing sex hormone secretion and testicular size. However, the degeneration of the KS testes accelerates dramatically at the time of puberty with complete hyalinization of the seminiferous tubules and Leydig cell hyperplasia in adulthood (for review see (1)). At present, it is unknown at what age hypogonadism can be biochemically detected in KS.

It is well established that the hypothalamic–pituitary–gonadal (HPG) axis is transiently activated during the first months of postnatal life. In the healthy male infant gonadotrophins, sex steroids and inhibin B increase to pubertal or even adult levels at ~3 months of age, followed by a relatively quiescent period until reactivation of the HPG axis in puberty (2–6). The elevated inhibin B persists for a longer period of time than the elevated follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (5, 7). The initial activation of the HPG axis is believed to be important for genital development, including renewal and differentiation of the germ cells. Furthermore, penile size (8) as well as postnatal testicular descent in cryptorchid boys (9) is influenced by sex steroids.

The postnatal surge of sex hormones represents a diagnostic window which enables the clinician to evaluate testicular function in infancy and to evaluate pituitary–testicular function in patients with congenital hypogonadotrophic hypogonadism (6, 10) as well as congenital adrenal hypoplasia (11), androgen insensitivity (12), Prader–Willi (13) and KS (14, 15).

To our knowledge, two studies have evaluated infant reproductive hormone levels in KS (14, 15). In both of these studies, it was shown that the testicular hormonal surge generated during the first months of postnatal life was compromised, with reduced testosterone (T) concentrations but normal serum levels of inhibin B (14), LH and FSH, suggesting some degree of primary testicular failure already present in the infancy period.

The aim of the present study was therefore to evaluate if primary testicular failure in KS is already present in early infancy.

DOI: 10.1530/EJE-07-0310
Online version via www.eje-online.org

Subjects and methods

Patients and controls

Ten boys with non-mosaic 47,XXY KS were investigated at the age of 3.1 months (range 1.8–3.8). The syndrome was diagnosed prenatally by amniocentesis as part of a screening programme and should therefore constitute an unbiased cohort with no selection through clinical symptoms. Diagnosis was confirmed by postnatal karyotyping. All boys had spontaneously descended testes at birth. Clinical data on the KS infants were obtained from patient records for this study.

The controls constituted 613 healthy boys aged 3.0 months (range 2.0–4.5) who participated in a prospective cohort study performed from 1997 to 2001 at the University Hospitals in Copenhagen, Denmark (Rigshospitalet and Hvidovre Hospital) (16). In this study, only boys with no signs of cryptorchidism, hypospadias or foreskin malformations were included.

Ethics

The study was performed according to the Helsinki II declaration and was approved by the local ethics committee ((KF) 01-030/97). Parents gave written informed consent. Registration of clinical data was approved by the Danish Data Protection Agency (1997-1200-074) and (2005-41-5479).

Hormonal assays

Blood samples were drawn from an antecubital vein at a median time of 1120 h (1040–1700 h) in the Klinefelter boys and at a median time of 1200 h (0900–1600 h) in the control group. Blood was clotted, centrifuged, and serum was stored at −20 °C until hormone analyses were performed. FSH and LH were measured by two-sided time-resolved immunofluorometric assays (Delfia; Wallac, Turku, Finland) with detection limits of 0.05 IU/l and intra- and inter-assay coefficients of variation (CV) of <3 and $<5\%$ respectively.

Serum inhibin B was measured by a specific ELISA (Oxford Bio Innovation, Oxford, UK) with detection limit of 20 pg/ml. Intra- and inter-assay CVs were <13 and 19% respectively.

Sex hormone-binding globulin (SHBG) was determined by a time-resolved fluoroimmunoassay (Delfia; Wallac) with a detection limit of 0.23 nmol/l and intra- and inter-assay CVs of $<5\%$.

Testosterone was measured by a solid-phase RIA (Coat-a-count; Diagnostic Products Corp., Los Angeles, CA, USA) without previous extraction. The detection limit was 0.23 nmol/l, and the intra- and inter-assay CVs were $<10\%$ at the highest levels and 17% at the lowest levels found.

Free testosterone (free T) was calculated using the equation of Vermeulen (17) and an assumed fixed albumin concentration. This assumption is based on the fact that changes in albumin have only minute effects on the calculated free T, and that it is reasonable not to measure individual albumin levels when abnormality is not suspected. We applied a fixed albumin concentration of 40 g/l in accordance with the age of the individuals (18).

FSH and LH levels were available in all KS infants, but inhibin B levels were only available in seven KS infants, and the free T concentration was only calculated in eight KS infants due to lack of SHBG measurements in two infants. In the control infants, hormone determinations were available in 609 (T), 602 (free T), 588 (LH), 591 (FSH), 607 (SHBG) and 578 (inhibin B) boys respectively.

Clinical evaluation

Body length was measured with a portable infantometer (Kiddimeter; Raven Equipment, Essex, UK) to the nearest 0.1 cm. Body weight was measured on a digital scale to the nearest 0.005 kg. Height was available in 543 controls, whereas weight was available in 577 controls. Testicular volume was evaluated by standard orchidometer measurements in the KS infants, but not the controls.

Statistical analysis

Biochemical characteristics are provided as medians, 2.5 and 97.5 percentiles, if not otherwise indicated.

Bivariate reference charts of total and free T in conjunction with the corresponding LH and inhibin B with corresponding FSH were constructed based on the control subjects as previously described (19–21). The bivariate reference represents a curve, beyond which a paired measurement of, for example, T and LH in an individual will be considered abnormal. These curves separate the individuals with an abnormal LH–T combination (or LH-free T or inhibin B–FSH) from the individuals with a normal combination. At the same time, one can read the 2.5 and 97.5 percentiles of the involved hormones as the horizontal and vertical lines respectively, of the curve. Thus, individuals outside the reference curve may have elevated serum levels of LH or FSH and/or decreased serum levels of testosterone or inhibin B, depending on the reference chart chosen.

The Mann–Whitney U test was used to test for differences in hormone levels between KS infants and controls. A P value of <0.05 was considered statistically significant.

Results

Clinical evaluation

Birth weight and clinical characteristics at the examination are presented in Table 1. There was no difference between birth weights between KS and controls, but KS infants were significantly lighter and shorter than controls at the time of examination. Testicular volume

Table 1 Clinical and biochemical characteristics of the Klinefelter infants and the healthy controls.

	Klinefelter infants $n=10$	Healthy controls $n=613$	*P* values
Birth weight (g)	3570 (2860–3900)	3623 (2436–4666)	NS
Age at examination (months)	3.1 (1.8–3.8)	3.0 (2.0–4.5)	NS
Height at examination (cm)	60.3 (56.0–64.3)	62.2 (58.2–66.9)	0.02
Weight at examination (kg)	6.1 (4.8–7.0)	6.5 (5.1–8.2)	0.04
Testosterone (nmol/l)	5.0 (2.2–11.2)	3.4 (0.7–8.3)	0.02
Free testosterone (pmol/l)	31.6 (18.2–61.8)	22.1 (4.3–48.4)	0.01
LH (IU/l)	3.3 (1.3–4.6)	1.7 (0.6–4.3)	0.005
FSH (IU/l)	1.7 (1.1–4.1)	1.2 (0.4–3.0)	0.007
Inhibin B (pg/ml)	330.0 (245.0–444.0)	387.0 (233.5–634.0)	NS
SHBG (nmol/l)	137.5 (83.0–188.0)	138.0 (66.2–269.2)	NS
LH/testosterone ratio	0.55 (0.17–2.1)	0.54 (0.17–2.3)	NS
LH/free testosterone ratio	83.3 (30.7–206.5)	82.3 (26.5–319.6)	NS
FSH/inhibin B ratio	6.5 (2.7–16.9)	3.0 (0.78–11.4)	0.005

P values represent comparison between KS infants and healthy controls using the Mann–Whitney *U* test. Results shown as median and 2.5 and 97.5 percentiles. NS, not significant.

was smaller (1.0 ml (0.5–2.0)) than previously reported by Cassorla *et al* (22) in healthy boys at 3 months of age (mean volume of left testicle 2.05 ml ($\pm$0.15 S.E.M.) and right testicle 1.95 ml ($\pm$0.11 S.E.M.)).

Biochemical evaluation

Basal serum levels of total T, free T, LH, FSH, SHBG and inhibin B in the KS infants according to age are shown in comparison with the levels of healthy control infants (Fig. 1A–F).

Bivariate evaluations of the relationships between corresponding levels of LH and T, LH and free T, and FSH and inhibin B respectively, are shown in Fig. 2. All but two KS infants were within the normal limits in the evaluation of both LH versus T and FSH versus inhibin B, whereas one KS infant was outside the limit in LH versus free T.

Discussion

We found high normal serum levels of total and free T and elevated levels of gonadotrophins in 3-month-old Klinefelter infants when compared to healthy controls. The boys of our study did not thereby have any biochemical signs of early hypoandrogenism.

The results of our study are in contrast with previously reported data on KS infants in which the levels of T compared to a control group were decreased, but LH, FSH and inhibin B (14) levels were reported to be normal (14, 15). However, when we compare the T levels of the 12 KS boys <6 months of age in the study of Ross and co-workers (15) with the levels in our 613 healthy infants (15), we found no differences. It was not possible to carry out such a comparison with the KS boys in the study of Lahlou *et al.* (14) as no individual concentrations were given. The same normative data for reproductive hormone levels in healthy infant boys (examined due to non-endocrine and non-metabolic reasons) were used in these two studies. This could be due to differences in hormone levels according to different assay platforms (23). Nevertheless, the reported levels of T in the control infants in the two KS studies were much higher (4–20 nmol/l (5th–95th percentiles); (14, 15)) than reported by any other study (2, 3, 5, 24–28), including this study.

Our study provides new data on the relationship between total T (and free T) and the corresponding LH values and between inhibin B and the corresponding FSH in 3-month-old infants. These bivariate evaluations of two related markers are valuable tools in adult andrological medicine (21). The two-dimensional reference charts visually describe the status of the pituitary–Leydig cell axis and the pituitary–Sertoli cell axis in normal infants by separating the individuals with an abnormal LH–T combination (or LH-free T or inhibin B–FSH) from the individuals with a normal combination.

In the bivariate LH–T evaluation, the majority of KS infants were inside the reference range although the levels were skewed. Concomitantly, high LH and T levels suggested an alteration in the pituitary–Leydig cell set point.

Our finding of increased FSH/inhibin B ratio suggests that a subtle Sertoli cell dysfunction might already be manifest at this early stage. Theoretically, increased gonadotrophin-releasing hormone drive due to the impaired Sertoli cell function may not only result in increased FSH but also increased the LH. However, inhibin B levels were only available in seven out of ten Klinefelter infants, and this statement needs further studies to be clarified.

The hormonal surge is known to arise during the first months of life and the diagnostic window is quite narrow, around 3 months of age. Our controls were examined between 2.0 and 4.5 months of age, whereas the Klinefelter boys were aged 1.8–3.8 months at examination. Although there was no difference between

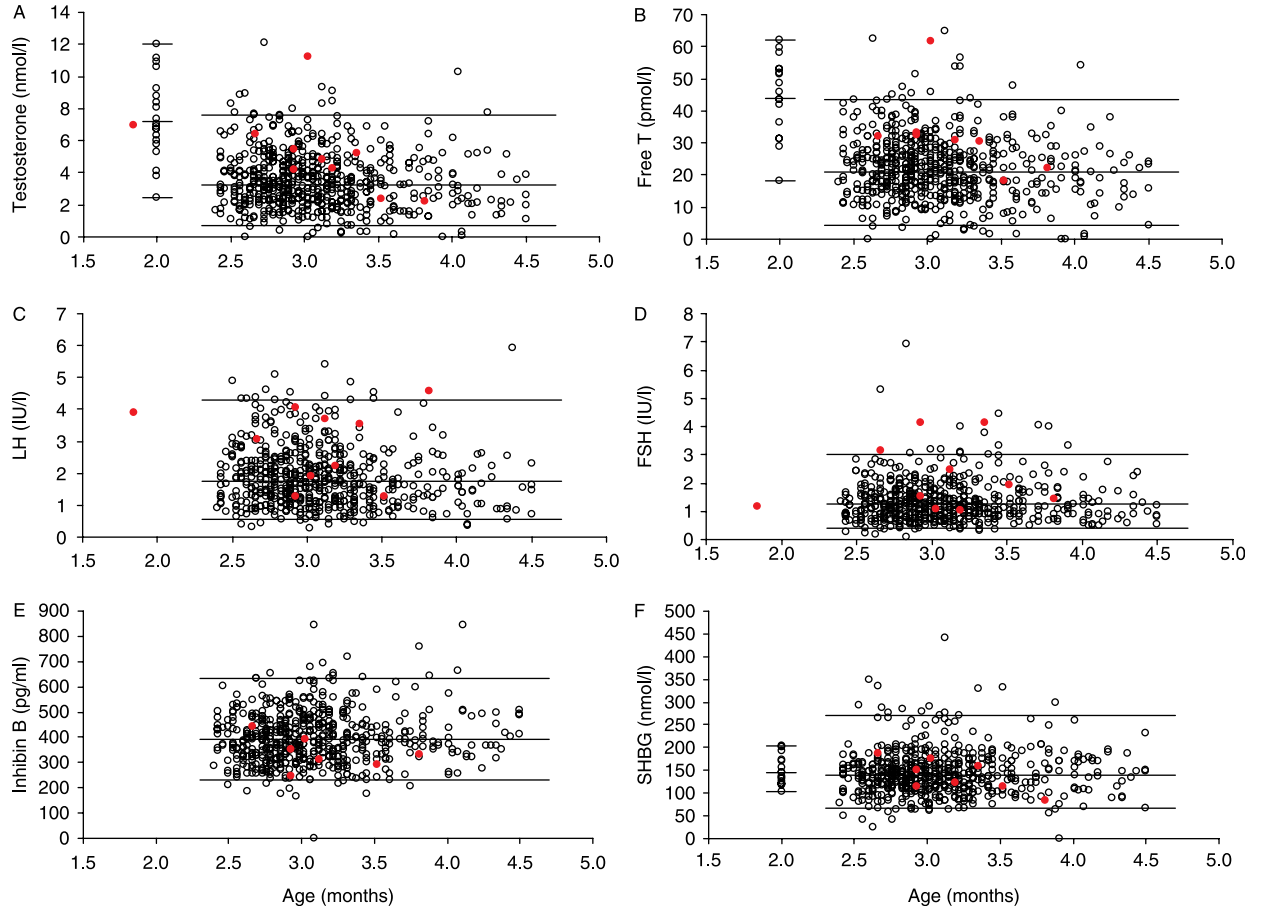

Figure 1 Serum total testosterone (A), free testosterone (B), LH (C), FSH (D), inhibin B (E) and SHBG (F) levels according to chronological age in ten infants with Klinefelter's syndrome (red dots) and 613 healthy control boys (open dots). Lines represent mean, 2.5 and 97.5 percentiles in healthy infants (16).

mean ages in the two groups, it can be speculated that the older controls bias the results. However, limiting the comparison to the controls of an age between 2.0 and 3.8 months did not alter any results (data not shown).

The incidence of cryptorchidism was recently found to be 6.25% higher in KS than in healthy controls (29). Diagnosis of KS was completed prenatally and our cohort of Klinefelter infants should therefore constitute an unbiased cohort. However, the present Klinefelter infants may not represent the broad phenotypic spectrum of KS usually followed in a paediatric endocrine clinic as none were born with cryptorchidism.

In a recent longitudinal study during puberty in KS, no signs of hypogonadism were evident until mid-puberty (30). These Klinefelter boys entered puberty at the expected age with increasing T secretion and showed normal changes in indices of androgen action (i.e. serum SHBG, leptin and prostate-specific antigen) (30). In that study, T secretion remained normal throughout puberty, whereas gonadotrophins increased to supranormal levels around mid-puberty. Thus, these boys seem to enter a stage of compensated hypoandrogenism after an apparently normal initiation of puberty.

Testosterone substitution is widely used in adults with KS, but it is unknown at present if early initiation of androgen replacement therapy at the onset of puberty would prevent symptoms of hypogonadism, i.e. osteoporosis, the metabolic syndrome, etc. Recently, androgen treatment in KS boys already in the infancy period has been suggested and case reports of such treatment exist (14). Future controlled studies on possible beneficial effects of T replacement in KS infants remain to be seen. However, our finding of normal (or even high normal) T in KS infants does not support routine androgen treatment in infancy of all KS infants.

In conclusion, our study of ten infants with KS provides evidence of a subtle Sertoli cell dysfunction with low normal inhibin B levels, but high normal levels of T and increased LH at this early age. We found no biochemical support for early hypoandrogenism, as has been reported previously.

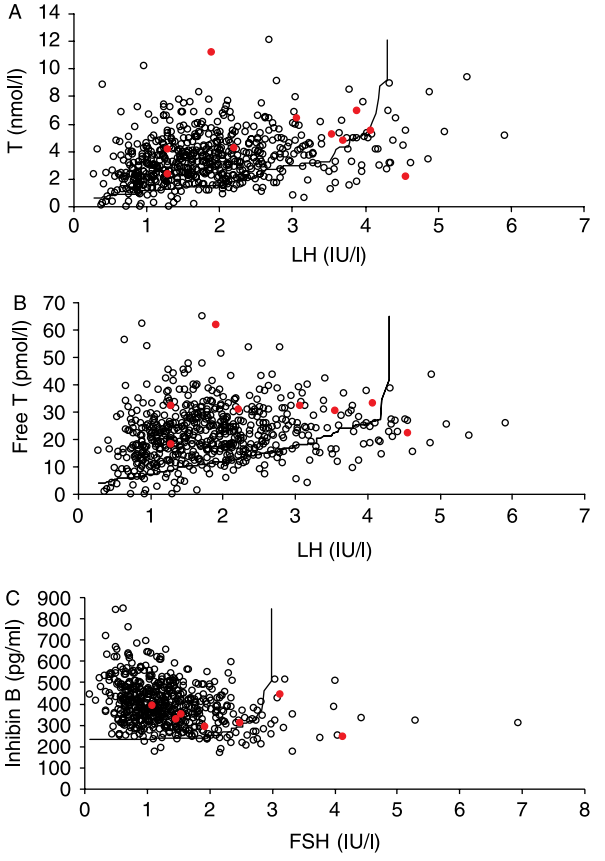

Figure 2 Bivariate reference charts of total testosterone (A) and free testosterone (B) according to corresponding LH levels and inhibin B (C) according to the corresponding FSH levels. The reference lines are based on hormone levels in the 613 healthy control infants aged 3.0 months. The vertical line of the curve is the 97.5 percentile of LH and FSH respectively. Similarly, the horizontal line represents the 2.5 percentile of T and inhibin B respectively (16, 19). Thus, for any point outside the reference curve for say LH–T, <2.5% of controls have a combination of higher LH and lower T. Red dots represent Klinefelter infants, while open dots represent the values of the control infants.

Acknowledgements

We wish to thank the members of the Nordic Cryptorchidism Study Group who performed all the examinations of the control boys.

References

1 Aksglaede L, Wikstrom M, Meyts ER, Dunkel L, Skakkebaek E & Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. *Human Reproduction Update* 2006 **12** 39–48.
2 Forest MG, Sizonenko PC, Cathiard AM & Bertrand J. Hypophysogonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *Journal of Clinical Investigation* 1974 **53** 819–828.
3 Winter JS, Hughes IA, Reyes FI & Faiman C. Pituitary–gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to two years of age. *Journal of Clinical Endocrinology and Metabolism* 1976 **42** 679–686.
4 Corbier P, Edward DA & Roffi J. The neonatal testosterone surge: a comparative study. *Archives Internationales de Physiologie, de Biochimie et de Biophysique* 1992 **100** 127–131.
5 Andersson AM, Toppari J, Haavisto AM, Petersen JH, Simell T, Simell O & Skakkebaek NE. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 675–681.
6 Grumbach MM. A window of opportunity: the diagnosis of gonadotropin deficiency in the male infant. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 3122–3127.
7 Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV & Kelnar CJ. Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. *Clinical Endocrinology* 2002 **56** 215–221.
8 Boas M, Boisen KA, Virtanen HE, Kaleva M, Suomi AM, Schmidt IM, Damgaard IN, Kai CM, Chellakooty M, Skakkebaek NE, Toppari J & Main KM. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *European Journal of Endocrinology* 2006 **154** 125–129.
9 Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, Skakkebaek NE & Toppari J. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 2004 **363** 1264–1269.
10 Main KM, Schmidt IM & Skakkebaek NE. A possible role for reproductive hormones in newborn boys: progressive hypogonadism without the postnatal testosterone peak. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 4905–4907.
11 Kaiserman KB, Nakamoto JM, Geffner ME & McCabe ER. Minipuberty of infancy and adolescent pubertal function in adrenal hypoplasia congenita. *Journal of Pediatrics* 1998 **133** 300–302.
12 Bouvattier C, Carel JC, Lecointre C, David A, Sultan C, Bertrand AM, Morel Y & Chaussain JL. Postnatal changes of T, LH, and FSH in 46,XY infants with mutations in the *AR* gene. *Journal of Clinical Endocrinology and Metabolism* 2002 **87** 29–32.
13 Fillion M, Deal CL & Van VG. Normal minipuberty of infancy in boys with Prader-Willi syndrome. *Journal of Pediatrics* 2006 **149** 874–876.
14 Lahlou N, Fennoy I, Carel JC & Roger M. Inhibin B and anti-Mullerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 1864–1868.
15 Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF & Zinn A. Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. *Hormone Research* 2005 **64** 39–45.
16 Main KM, Toppari J, Suomi AM, Kaleva M, Chellakooty M, Schmidt IM, Virtanen HE, Boisen KA, Kai CM, Damgaard IN & Skakkebaek NE. Larger testes and higher inhibin B levels in Finnish than in Danish newborn boys. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2732–2737.
17 Vermeulen A, Verdonck L & Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology and Metabolism* 1999 **84** 3666–3672.
18 Stapleton RD & Plotnik L. Diagnostic and therapeutic information, Part II. In *The John Hopkins Hospital: The Harriet Lane Handbook*, edn 13, Ed. KB Johnson. St. Louis: Mosby, 1993.
19 Petersen JH. Two bivariate geometrically defined reference regions with applications to male reproductive hormones and human growth. *Statistics in Medicine* 2003 **22** 2603–2618.
20 Andersson AM, Petersen JH, Jorgensen N, Jensen TK & Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: significance of adequate reference values from proven fertile men. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 2873–2879.
21 Aksglaede L, Andersson AM, Jorgensen N, Jensen TK, Carlsen E, McLachlan RI, Skakkebaek NE, Petersen JH & Juul A. Primary

testicular failure in Klinefelter's syndrome: the use of bivariate luteinizing hormone-testosterone reference charts. *Clinical Endocrinology* 2007 **66** 276–281.

22 Cassorla FG, Golden SM, Johnsonbaugh RE, Heroman WM, Loriaux DL & Sherins RJ. Testicular volume during early infancy. *Journal of Pediatrics* 1981 **99** 742–743.

23 Sikaris K, McLachlan RI, Kazlauskas R, de Kretser D, Holden CA & Handelsman DJ. Reproductive hormone reference intervals for healthy fertile young men: evaluation of automated platform assays. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 5928–5936.

24 Bergada I, Milani C, Bedecarras P, Andreone L, Ropelato MG, Gottlieb S, Bergada C, Campo S & Rey RA. ime course of the serum gonadotropin surge, inhibins, and anti-Mullerian hormone in normal newborn males during the first month of life. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 4092–4098.

25 Forest MG, De PE & Bertrand J. Hypothalamic–pituitary–gonadal relationships in man from birth to puberty. *Clinical Endocrinology* 1976 **76** 551–569.

26 Forest MG, De PE & Bertrand J. Testicular and adrenal androgens and their binding to plasma proteins in the perinatal period: developmental patterns of plasma testosterone, 4-androstenedione, dehydroepiandrosterone and its sulfate in premature and small for date infants as compared with that of full-term infants. *Journal of Steroid Biochemestry* 1980 **12** 25–36.

27 Burger HG, Yamada Y, Bangah ML, McCloud PI & Warne GL. Serum gonadotropin, sex steroid, and immunoreactive inhibin levels in the first two years of life. *Journal of Clinical Endocrinology and Metabolism* 1991 **72** 682–686.

28 Gendrel D, Chaussain JL, Roger M & Job JC. Simultaneous postnatal rise of plasma LH and testosterone in male infants. *Journal of Pediatrics* 1980 **97** 600–602.

29 Bojesen A, Juul S, Birkebaek N & Gravholt CH. Morbidity in Klinefelter syndrome: a Danish Register Study Based on Hospital Discharge Diagnoses. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 1254–1260.

30 Wikstrom AM, Dunkel L, Wickman S, Norjavaara E, Ankarberg-Lindgren C & Raivio T. Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47,XXY boys *Pediatric Research* 2006 **59** 854–859.

Received 11 May 2007
Accepted 20 June 2007