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ORIGINAL ARTICLE

Pubertal testicular volume references for ruler, orchidometer, and ultrasonography measurements based on a longitudinal follow-up

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Abstract

Background: Testicular volume is a marker of male pubertal development. Various clinical conditions and their treatments may influence testicular growth.

Objectives: To create ruler-based age-dependent pubertal testicular volume references that enable calculation of standard deviation (SD) scores.

Materials and Methods: Study cohort comprised 65 boys who attended clinical examination twice a year from the age of 8.5 years until the attainment of final testicular size. Forty-nine (75.4%) boys completed the follow-up and 16 (24.6%) boys dropped out before the attainment of final post-pubertal testicular size. At each follow-up visit testicular size was measured with a ruler, orchidometer, and ultrasonography. LMS or LMSP method served as the technique for creating reference growth curves for testicular volumes. Using the novel references for ruler measurements, development of SD scores was assessed in a cohort of boys with unilateral cryptorchidism.

Results: Reference growth curves were constructed separately for ruler, orchidometer, and ultrasonography measurements. Median orchidometer volume of 4 mL, marker of male pubertal onset, occurred at the age of 11.7 years, whereas +2SD curve surpassed 4 mL at 10.2 years and −2SD curve at 13.7 years. Modeled ages at the attainment of 4 mL testicular volume based on ruler measurements were 9.7 years for +2SD curve, 11.5 years for median curve, and 13.6 years for −2SD curve. Ultrasonography-based volume of 1.3 mL corresponded with the median modeled orchidometer-based volume of 4 mL. In boys with unilateral cryptorchidism, ruler-based SD scores decreased during puberty in undescended testes, but not in descended testes.

Discussion and Conclusion: The present study provides reference values for pubertal testicular volume measured with a ruler enabling an age-dependent assessment of testicular size. Comparison with measurements by an orchidometer and ultrasonography is also presented.

KEYWORDS
cryptorchidism, ellipsoid formula, growth curves, ultrasound, Z score

1 INTRODUCTION

Testicular volume is an important marker of male pubertal development and fertility in clinical practice and research as it correlates with Sertoli cell quantity and semen quality.[1–4] Elongation of the seminiferous cords is the main cause of testicular enlargement before puberty. After pubertal onset, testicular growth occurs due to dilatation of the seminiferous tubules caused by proliferation of Sertoli cells and spermatogonia.[5] Pubertal onset is caused by an activation of the hypothalamus–pituitary–testis axis which results in an increased secretion of follicle-stimulating hormone and luteinizing hormone. This eventually triggers spermatogenesis and testosterone production in the testes. However, inter-individual variability during puberty is wide, and testicular volume is typically the first assessment for precocious or delayed puberty in boys.[6]

Various conditions and their treatments, such as cryptorchidism, Klinefelter syndrome, and treatment of childhood cancer, may influence testicular development during puberty.[7–9] Reference values for normal pubertal testicular volumes would provide tools for timely detection of impaired testicular growth in individual patients. Moreover, in clinical research, they would provide an opportunity to evaluate, for example, treatment-related effects on pubertal testicular growth. Age-specific testicular volume references exist for orchidometer and ultrasonography.[10,11] Probably due to its availability, measurement of testicular dimensions with a ruler is also an applied method for assessing testicular volume.[12–14] However, for ruler there are currently no growth references available.

We aimed to construct pubertal testicular reference growth charts for testicular volume based on ruler measurements to enable calculation of age-dependent standard deviation (SD) scores. We also assessed the performance of the novel ruler-based growth charts of testicular volume in cohorts of boys with cryptorchidism[15] and Klinefelter syndrome.[12] In addition, to compare their relation to the ruler-based growth charts, we constructed growth charts for testicular volume based on Prader orchidometer and ultrasonography.

2 MATERIALS AND METHODS

2.1 Study design and study cohort description

The study cohort included 65 healthy boys who participated in a pubertal follow-up study which took place in the University of Turku, Finland, between August 2005 and September 2019. The pubertal follow-up consisted of biannual follow-up visits from the age 8.5 years until the attainment of final post-pubertal testicular size, defined as the lack of testicular growth in three consecutive follow-up visits.[15,16] The study cohort was recruited from a prospective birth cohort study assessing prevalence and risk factors of congenital cryptorchidism.[17] Detailed participant recruitment process is described in Figure 1. For the pubertal follow-up study, the study cohort (i.e., healthy boys) was originally recruited as a control group for a cohort of boys with history of cryptorchidism; two previous publications have compared pubertal testicular growth (based on ultrasonography) between healthy boys and boys with history of cryptorchidism.[15,16] This is, however, the first time we report ruler-based reference values for normal pubertal testicular growth.

2.2 Measurements

Testicular size was measured with Prader orchidometer, plastic ruler, and ultrasonography (Aloka Prosound 6, linear probe, 5–13 MHz and Aloka SSD-500, linear probe, 7.5 MHz; Hitachi Aloka Medical) at each follow-up visit. Our study cohort represents healthy boys with no testicular pathology. Therefore, mean volume of right and left testicle was used in the analyses. Orchidometer contained a set of 12 ellipsoid beads representing 12 volumes between 1 and 25 mL. Examiner was also allowed to estimate volumes that were between the beads or greater than 25 mL. At each visit testicular dimensions (length and width) were measured once with ruler and three times with ultrasonography. For ultrasonography-based testicular length and width, mean values were included in the analyses. Ellipsoid formula ($\pi/6 \times$ length (L) $\times$ width (W)^2) was used when calculating testicular volumes based on ruler and ultrasonography measurements. Data contained only five testicular volume measurements after the 17.5-year follow-up visit; these were excluded from the analyses because, due to the limited number, they would not have provided reliable estimates. For boys who attained final post-pubertal testicular size before the age of 17.5 years, the values from the subsequent missing biannual follow-up visits were imputed until the age of 17.5. Rationale for the imputation was to obtain a balanced cohort at the time of all follow-up visits. Imputed values were defined as the mean of the volumes measured at the last two follow-up visits. No imputation was conducted for boys who dropped out of the study or attained final testicular size at the age of 17.5 years or later. The boys who dropped out reported that they were uninterested to continue. In addition to testicular size, Tanner P stage was evaluated at each follow-up visit.[18]

2.3 Validation cohorts

The novel growth charts of testicular volume based on ruler measurements were evaluated with testicular volume data from other boy

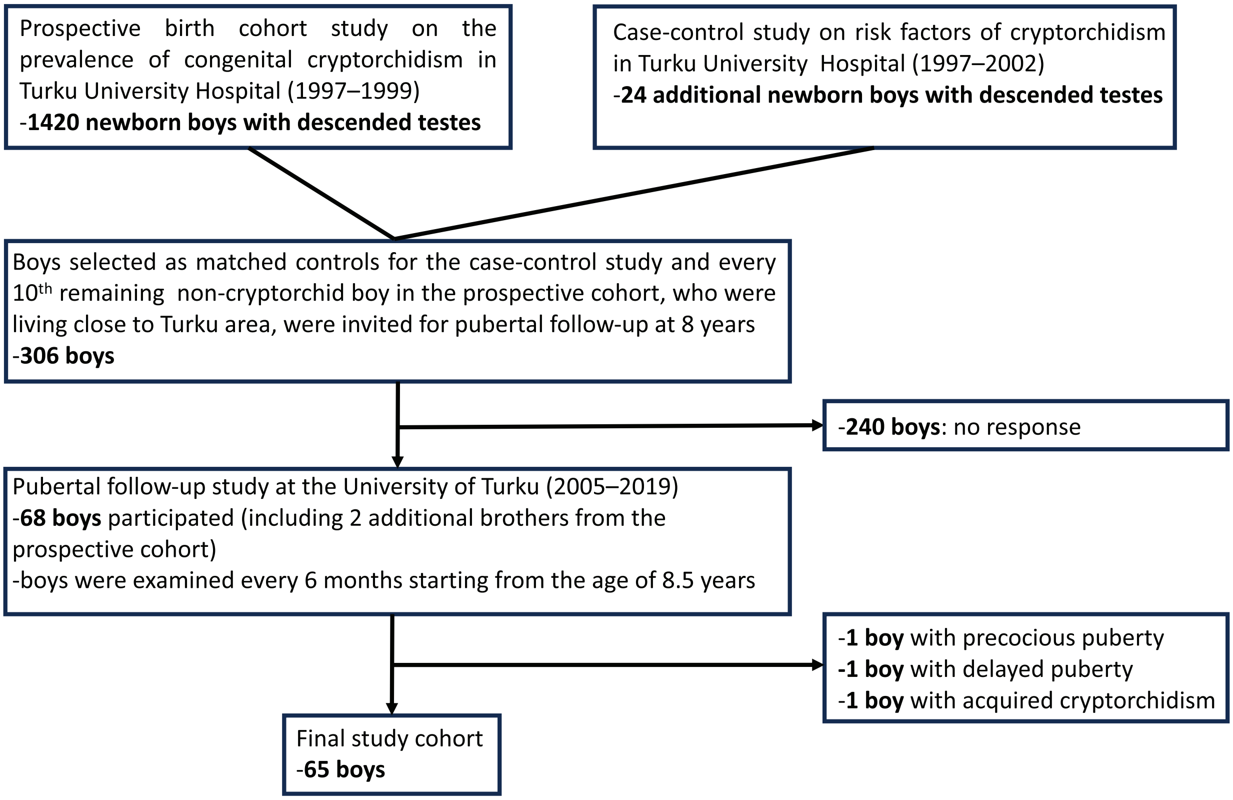

FIGURE 1 Flow chart of the participant recruitment process.

cohorts: boys with a history of unilateral cryptorchidism ($n = 30$)[15] and boys with Klinefelter syndrome ($n = 14$).[12] The boys with a history of unilateral cryptorchidism had undergone similar biannual pubertal (from age 8.5 years until the attainment of final post-pubertal testicular size) follow-up protocol as the study cohort. In the validation analyses, we analyzed the longitudinal development of testicular volume during puberty; descended and undescended testes were analyzed in separate groups. For the boys with Klinefelter syndrome, testicular volumes and corresponding ages were obtained from the original publication[12] using Web Plot Digitizer,[19] a web-based application enabling data extraction from graphical plots.

2.4 | Statistical methods

R statistical software (R Foundation for Statistical Computing) version 4.1.3 for Windows provided tools for statistical analyses. Growth curves were constructed with the generalized additive models for location, scale, and shape (GAMLSS) package using penalized B-splines (P-splines) in smoothing.[20] Even though our data cover ages 8.5–17.5, we estimated growth curves from 9 to 17 years to minimize the so-called edge effects, that is, erratic growth curve estimation at both extremes of the data.[21] Optimal degrees of freedom for the smoothing parameters were appraised with the Akaike information criteria (AIC). Besides AIC, model selection included analyses of observed versus fitted centiles, *Q*-statistics, and worm plots.

First, we modeled testicular growth during puberty using LMS method, in which time-varying average values and reference range is expressed with three parameters related to skewness (*L*), approximate median (*M*), and approximate coefficient of variation (*S*). The parameter values of *L*, *M*, and *S* at a specific age enable calculation of SD scores with the following equation: $(((\text{volume}/M)^{L}) - 1)/(L \times S)$, where volume is the measured testicular volume. If model diagnostics (analyses of observed vs. fitted centiles, *Q*-statistics, and worm plots) indicated insufficient fit in the LMS model, growth curves were constructed using the LMSP method, which also allows the modeling of a kurtosis-related parameter.[22,23] Eventually, growth curves were constructed with the LMS method for ruler-based measurements and with the LMSP method (i.e., Box–Cox power exponential [BCPE] distribution) for measurements based on orchidometer and ultrasonography.

2.5 | Ethics

The Joint Ethics Committee of the University of Turku and Turku University Hospital approved the birth cohort study and the case–control study (July 1996, update June 2001) and the pubertal follow-up study (December 2004). All participants and their parents provided written

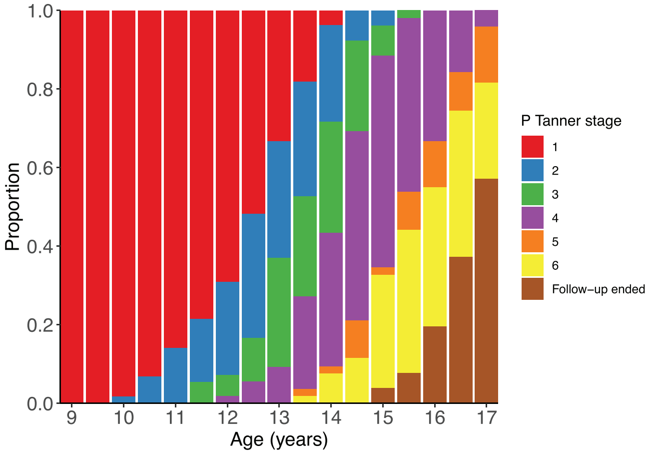

FIGURE 2 Distribution of Tanner P stages at each biannual follow-up visit in a cohort of 65 healthy boys.

informed consent. The study was conducted according to the Helsinki II Declaration.

3 | RESULTS

3.1 | Study cohort

The study cohort comprised 65 healthy boys with 947 testicular volume measurements between ages 8.5 and 17.5 years. The median number of study visits was 16 [interquartile range (IQR) 14–18)] and the median follow-up was 7.5 (IQR 6.5–8.5) years. In addition, 99 testicular volume measurements were imputed for boys who attained final testicular size before the age of 17.5 years. Of the orchidometer measurements (both testes combined, imputed values not included), 847 (44.5%) were judged as in-between two beads and 133 (7.0%) as over 25 mL. Table S1 provides the number of observations at each biannual visit. Forty-nine (75.4%) boys completed the follow-up and 16 (24.6%) boys dropped out before the attainment of final post-pubertal testicular size. The mean age at the attainment of final testicular volume was 16.6 (SD 1.0) years. The 16 boys who dropped out of the study provided 120 testicular volume measurements between the ages 8.5 and 16.5 years; the median age at the last follow-up visit before dropping out of the study was 10.8 (IQR 9.0–14.6) years. Figure 2 and Table S2 exhibit the development of Tanner P stages in puberty. Mean age at attainment of Tanner P stage ≥2 was 12.5 years (SD 1.2, range 10–14.5 years).

3.2 | Testicular volume growth charts

Figure 3 and Table 1 present age-specific reference values for testicular volumes measured by a ruler, orchidometer, and ultrasonography. Median modeled orchidometer volume of 4 mL occurred at the age of 11.7 years; +2SD curve surpassed 4 mL at 10.2 years and −2SD curve at 13.7 years. Correspondingly, modeled ages at the attainment of 4 mL testicular volume based on measurements with a ruler were 9.7 years for +2SD curve, 11.5 years for median curve, and 13.6 years for −2SD curve. The difference in the modeled −2SD ruler- and orchidometer-based curves was less than 0.6 mL throughout the pubertal growth (Figure 3D). However, estimated +2SD curves exhibited greater difference especially in late puberty. The estimated growth curve for ultrasonography-based testicular volume differed distinctly from the orchidometer- and ruler-based curves (Figure 3D). At the age (11.7 years) when median orchidometer-based testicular volume was 4 mL, modeled median ultrasonography-based volume was 1.3 mL. The +2SD ultrasonography-based volume curve crossed 1.3 mL at the age of 10.4 years and −2SD at the age of 13.5 years. Tables S3–5 provide comprehensive age-specific reference values in numerical form for ruler-, orchidometer-, and ultrasonography-based volume measurements, and Figures S1–3 show all observations superimposed to the reference curves. Table S6 provides LMS parameter values for the ruler-based model.

3.3 | Comparison to validation cohorts

When analyzing descended and undescended testes of subjects with unilateral cryptorchidism, median SD scores of descended testes were close to the median reference curve whereas median SD scores of undescended testes declined throughout puberty (Figure 4A,B). All SD scores of individual patients with Klinefelter syndrome were below the median growth curve and declined further approximately after the age of 13 years (Figure 4C).

4 | DISCUSSION

To our knowledge, we are the first to report age-specific reference values for testicular volumes during puberty based on ruler measurements. They are based on a longitudinal follow-up data of 65 healthy boys who underwent a clinical examination twice a year from the age of 8.5 years until the achievement of final post-pubertal testicular size. For comparison purposes, our study also includes growth charts for measurements based on orchidometer and ultrasonography. The novel growth charts can aid clinicians in identifying abnormalities in male pubertal development. Furthermore, in clinical research, main advantage of using SD scores is that analyses can involve patient cohorts of varying age distributions.

Testicular growth is a key event in male pubertal development. The pubertal testicular enlargement is caused mostly by an increase in germ cell numbers in the seminiferous tubular compartment because of the onset of spermatogenesis.[5] This, in turn, is caused by the secretion of androgens from the Leydig cells after the activation of the hypothalamic–pituitary–testicular axis.[5,24] In clinical practice, testicular volume is often measured in the evaluation of male infertility, or to assess pubertal development when precocious puberty, hypogonadotropic or hypergonadotropic hypogonadism, or delayed puberty are suspected.[4,6] Early studies by Prader and others suggest that the

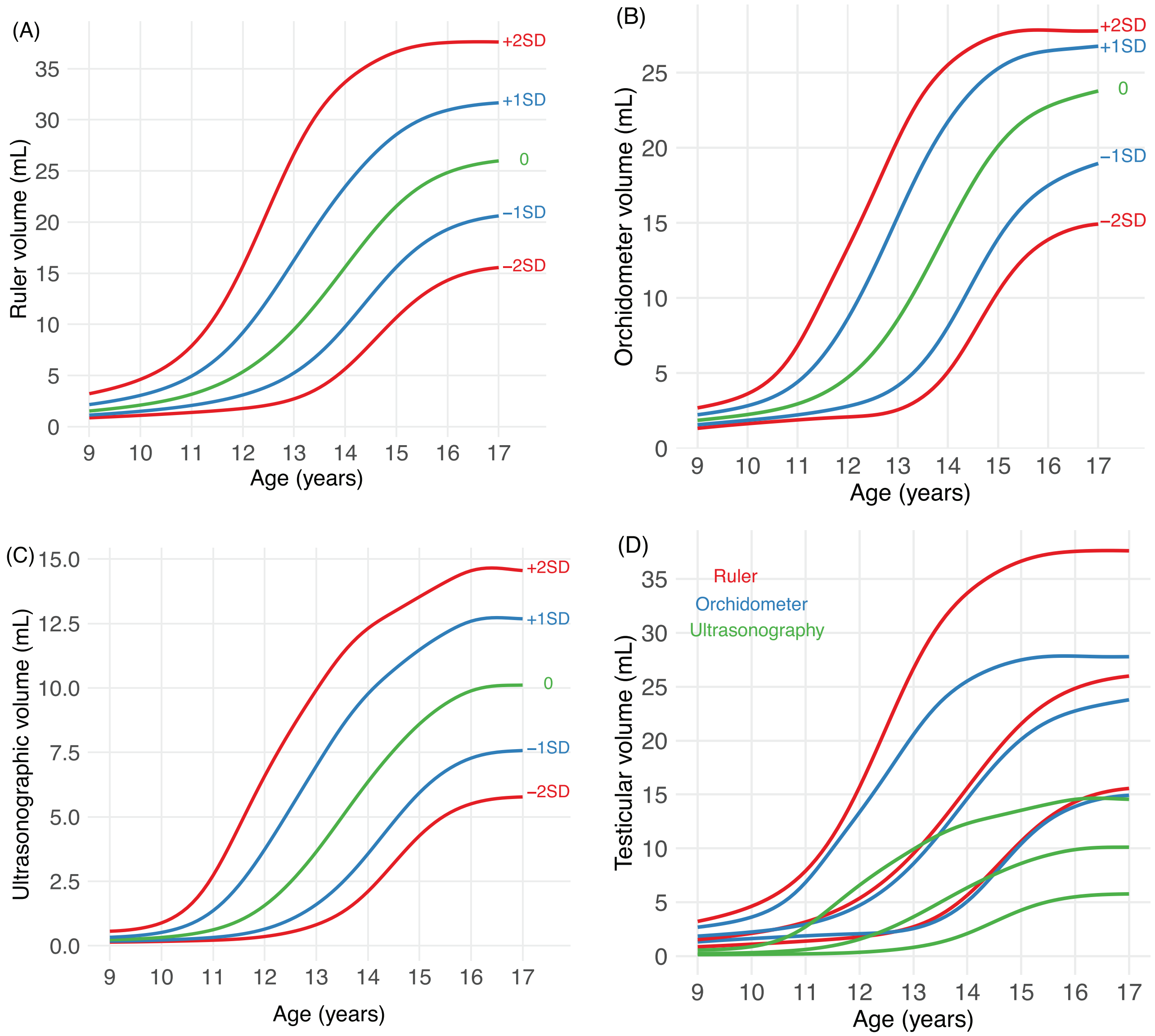

FIGURE 3 Testicular volume growth charts for (A) ruler measurements, (B) orchidometer measurements, and (C) ultrasonography measurements. (D) +2SD, 0, and −2SD curves for ruler, orchidometer, and ultrasonography measurements in one plot.

TABLE 1 Age-dependent reference values for testicular volume based on ruler, orchidometer, and ultrasonography measurements.

Age	Ruler			Orchidometer			Ultrasonography		
	−2 SD	0	+2 SD	−2 SD	0	+2 SD	−2 SD	0	+2 SD
9	0.9	1.5	3.2	1.3	1.9	2.7	0.14	0.23	0.56
10	1.1	2.1	4.6	1.6	2.2	3.6	0.17	0.32	0.89
11	1.4	3.2	7.9	1.9	3.0	6.8	0.21	0.62	2.7
12	1.8	5.4	15.7	2.1	4.7	13.3	0.36	1.6	6.6
13	2.7	9.5	26.7	2.6	8.6	20.5	0.81	3.6	9.9
14	5.7	15.6	33.7	5.1	14.6	25.5	2.1	6.4	12.3
15	10.7	21.6	36.6	10.4	20.1	27.5	4.3	8.6	13.5
16	14.3	24.8	37.6	13.9	22.8	27.8	5.5	9.9	14.5
17	15.6	26.0	37.6	14.9	23.8	27.8	5.8	10.1	14.6

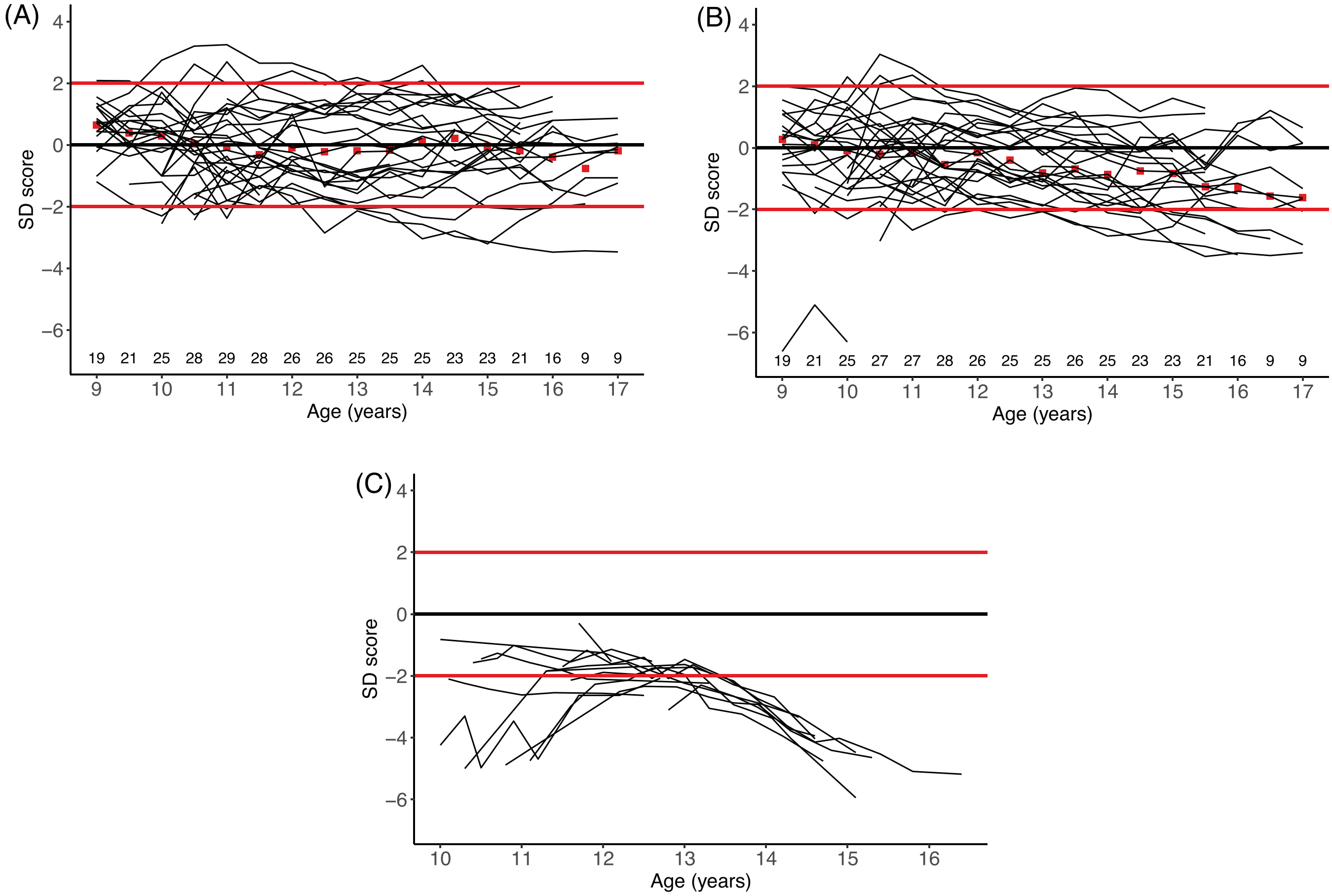

FIGURE 4 (A and B) Development of standard deviation (SD) scores for ruler-based testicular volume in individual patients with unilateral cryptorchidism: (A) descended testis and (B) undescended testis. Red lines denote −2SD and +2SD curves and black line denotes 0 curve. Red squares denote median SD score at each biannual follow-up visit. Numbers at the bottom of the figures denote the number of patients at each biannual follow-up visit. (C) Development of SD scores for ruler-based testicular volume in individual patients with Klinefelter syndrome.

attainment of testicular volume of 4 mL by Prader orchidometer marks the onset of puberty.[6] In addition, conceivably due to the equivalence with the length of the 4 mL orchidometer bead, some guidelines suggest that onset of puberty has occurred when ruler length of 25 mm is achieved.[25] Ultrasonography is probably more accurate in assessing testicular size,[26] but it requires more training and experience. It is rarely available in an outpatient visit and cannot be easily included in daily practice. Therefore, there is a need for a reliable ruler-based tool for assessment of age-adjusted testicular size. While ultrasonography measurement remains a gold standard for testicular assessment in clinical research, both a ruler and an orchidometer are practical tools in clinical evaluation during puberty.

Previously, Joustra et al. have reported ultrasonography- and orchidometer-based reference volumes from a cohort of Dutch boys.[11] In addition, from a Norwegian cohort, Oehme et al. have provided reference data for measurements based on ultrasonography.[10] However, both studies have some methodological differences compared with the present study. Main difference is in the formulas for testicular volume: volume was calculated with ellipsoid formula in the present study ($\pi/6 \times L \times W^2$) and in the study by Joustra et al. ($0.52 \times L \times W \times$ height (H)), whereas Oehme et al. used the Lambert formula ($0.71 \times L \times W \times H$). To enable comparison, the ultrasonography-based volumes from the study by Oehme et al. must be multiplied by $\frac{0.52}{0.71}$. Additionally, Joustra et al. and Oehme et al. measured three testicular diameters as recently advocated by the European Academy of Andrology,[27] whereas we measured only testicular length and width. Another difference is in the estimation of testicular sizes that appeared as intermediate between two orchidometer beads or larger than the 25 mL bead. However, the present orchidometer and ultrasonography-based testicular growth charts are comparable to the references assessed from the Dutch and Norwegian populations. Particularly estimates of the lower centiles (i.e., −2SD curve) are concordant and seem suitable for assessing male pubertal development in European populations. That is essential because the evaluation of impaired testicular growth relates to many clinically important questions. However, due to the limitations in our ultrasonographic measurements (lack of the third-dimension measurement), the present normal values may not be universally acceptable for the use in clinical practice.

Attainment of testicular size of 4 mL based on Prader orchidometer has traditionally marked the onset of puberty.[6] In our orchidometer-based model, this occurred at the median age of 11.7 years, which is consistent with other Western populations.[28,29] Median modeled age (11.5 years) at the attainment of ruler-based volume of 4 mL matched closely with the orchidometer model. Furthermore, to some extent the ruler-based reference curves followed the corresponding orchidometer-based curves. Ruler-based volumes can hence serve, at least in the lower centiles, as an approximation of the orchidometer-based testicular size. The upper centiles (+2SD) differed more, probably due to that with orchidometer it is difficult to estimate volumes greater than 25 mL. Consistent with previous studies,[11,26] modeled pre-pubertal and post-pubertal ultrasonography-based testicular volumes were lower than those derived from the orchidometer measurements. In ultrasonography, the adjacent soft tissue and epididymis have no influence on the measured testicular volume, unlike in assessments based on ruler or the Prader orchidometer.[26,30] In our study the ultrasonography-based volume of 1.3 mL corresponded with the median modeled orchidometer-based volume of 4 mL. This is in agreement with the findings by Joustra et al. who reported a correspondence between the 4 mL orchidometer size and ultrasonographic volume of 1.4 mL.[11]

The novel ruler-based reference charts were evaluated with additional testicular volume data. The SD scores of the descended testes of boys with unilateral cryptorchidism[15] performed appropriately as the median scores occurred near the median curve throughout puberty. Monitoring of testicular growth is essential particularly in patients with syndromes and conditions affecting pubertal/testicular development. The SD scores of the undescended testes of cryptorchid boys were comparable to those of descendent testes in pre-puberty. However, they exhibited a lag in growth throughout puberty which is consistent with preceding findings on reduced testicular volume in adult men with a history of cryptorchidism.[8,31] The age-dependent SD scores performed consistently also in patients with Klinefelter syndrome: all patients underwent a similar decline in SD scores, which probably reflects the previously reported deterioration of spermatogenesis before adolescence.[32]

The World Health Organization (WHO) published in 2006 worldwide applicable standards for the monitoring of child growth.[33] As part of the study, WHO-organized expert group reviewed 30 growth curve construction methods, and LMSP method (BCPE distribution) was ultimately chosen as the technique for creating the reference curves from the WHO cohort that combined longitudinal and cross-sectional data.[21] LMS method and its extensions have subsequently become conventional techniques for producing referential growth curves.[10,11,34–36] In the present study, reference charts were constructed with the LMS method for ruler-based measurements and with the LMSP method for orchidometer and ultrasonography. Due to the study design the growth curves extend only to the age of 17 years. As in few boys the follow-up was still ongoing at that time, adult testicular volumes cannot be estimated from the present references.

Our study has limitations. First, assessment of testicular volume may have noteworthy interobserver variation. This applies particularly to orchidometer,[37] whereas in a Norwegian study no significant interobserver variation occurred in testicular ultrasound measurements.[38] Little is known of interobserver variation when assessing testicular volume by a ruler. Second, due to the sample size of 65 longitudinally followed boys, the number of observations were limited beyond the limits of −2SD and +2SD curves. Therefore, SD scores below −2SD and over +2SD should be interpreted with caution. Third, orchidometer-based testicular volume is a discrete variable but is treated as a continuous one in the growth curves. Thus, interpretation of model values between two orchidometer beads is less clear. Fourth, the European Academy of Andrology recommends measurement of three testicular dimensions in ultrasonographic assessment,[27] but the present growth charts are based on measurement of testicular length and width. Fifth, our median growth curves may not approximate the shape of testicular growth in individuals. At most time points our median growth curves include a mixture of individuals who are in the middle of the intensive testicular growth as well as pre-pubertal and post-pubertal individuals. Consequently, the duration of the growth may be more prolonged and growth less steep in the median curves than in single individuals.

The strengths of our study include the biannual follow-up protocol with testicular volume measured with a ruler, orchidometer, and ultrasonography. The tight follow-up ensured that we were able to capture the rapid development and growth that occurs during puberty. Additionally, as testicular size was assessed with the three methods concurrently, relative difference between the methods could be assessed accurately. Another strength is that roughly three quarters of the study cohort completed the follow-up protocol. As a result, we have sufficient data at each follow-up visit.

In conclusion, the present longitudinal study provides reference growth charts for testicular volume measured with a ruler. The growth charts and respective age-specific referential values allow an age-dependent assessment of testicular size and calculation of SD scores for individual testicular volumes which will be useful for pediatricians and clinical researchers.

AUTHOR CONTRIBUTIONS

Study design: Mikael Koskela, Helena E. Virtanen, Kirsi Jahnukainen, Jorma Toppari, and Jaakko J. Koskenniemi. Data curation: Wiwat Rodprasert. Data analyses: Mikael Koskela and Jaakko J. Koskenniemi. First draft of the manuscript: Mikael Koskela. All the authors participated in editing the manuscript and approved the final version.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Datasets generated and analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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REFERENCES

1. Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*. 2003;125(6):769-784.
2. Hart RJ, Doherty DA, McLachlan RI, et al. Testicular function in a birth cohort of young men. *Hum Reprod*. 2015;30(12):2713-2724.
3. Grinspon RP, Urrutia M, Rey RA. Male central hypogonadism in paediatrics—the relevance of follicle-stimulating hormone and Sertoli cell markers. *Eur Endocrinol*. 2018;14(2):67-71.
4. Lotti F, Frizza F, Balercia G, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: scrotal ultrasound reference ranges and associations with clinical, seminal, and biochemical characteristics. *Andrology*. 2021;9(2):559-576.
5. Koskenniemi JJ, Virtanen HE, Toppari J. Testicular growth and development in puberty. *Curr Opin Endocrinol Diabetes Obes*. 2017;24(3):215-224.
6. Tinggaard J, Mieritz MG, Sørensen K, et al. The physiology and timing of male puberty. *Curr Opin Endocrinol Diabetes Obes*. 2012;19(3):197-203.
7. van Santen HM, van de Wetering MD, Bos AME, Vd Heuvel-Eibrink MM, van der Pal HJ, Wallace WH. Reproductive complications in childhood cancer survivors. *Pediatr Clin North Am*. 2020;67(6):1187-1202.
8. Koch T, Hansen AH, Priskorn L, et al. A history of cryptorchidism is associated with impaired testicular function in early adulthood: a cross-sectional study of 6376 men from the general population. *Hum Reprod*. 2020;35(8):1765-1780.
9. Bonomi M, Rochira V, Pasquali D, Balercia G, Jannini EA, Ferlin A. Klinefelter syndrome (KS): genetics, clinical phenotype and hypogonadism. *J Endocrinol Invest*. 2017;40(2):123-134.
10. Oehme NHB, Roelants M, Saervold Bruserud I, et al. Reference data for testicular volume measured with ultrasound and pubic hair in Norwegian boys are comparable with Northern European populations. *Acta Paediatr*. 2020;109(8):1612-1619.
11. Joustra SD, van der Plas EM, Goede J, et al. New reference charts for testicular volume in Dutch children and adolescents allow the calculation of standard deviation scores. *Acta Paediatr*. 2015;104(6):e271-e278.
12. Wikström 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. *Pediatr Res*. 2006;59(6):854-859.
13. Raivio T, Huhtaniemi I, Anttila R, et al. The role of luteinizing hormone-beta gene polymorphism in the onset and progression of puberty in healthy boys. *J Clin Endocrinol Metab*. 1996;81(9):3278-3282.
14. Taskinen S, Taavitsainen M, Wikström S. Measurement of testicular volume: comparison of 3 different methods. *J Urol*. 1996;155(3):930-933.
15. Sadov S, Koskenniemi JJ, Virtanen HE, et al. Testicular growth during puberty in boys with and without a history of congenital cryptorchidism. *J Clin Endocrinol Metab*. 2016;101(6):2570-2577.
16. Rodprasert W, Koskenniemi JJ, Virtanen HE, et al. Reproductive markers of testicular function and size during puberty in boys with and without a history of cryptorchidism. *J Clin Endocrinol Metab*. 2022;107(12):3353-3361.
17. Boisen KA, Kaleva M, Main KM, et al. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet*. 2004;363(9417):1264-1269.
18. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;45(239):13-23.
19. Webplotdigitizer 4.5. Accessed April 2022. https://automeris.io/WebPlotDigitizer
20. Rigby RA, Stasinopoulos DM. Generalized additive models for location, scale and shape (with discussion). *Appl Stat*. 2005;54:507-554.
21. Borghi E, de Onis M, Garza C, et al. Construction of the World Health Organization child growth standards: selection of methods for attained growth curves. *Stat Med*. 2006;25(2):247-265.
22. Cole TJ, Green PJ. Smoothing reference centile curves: the LMS method and penalized likelihood. *Stat Med*. 1992;11(10):1305-1319.
23. Stasinopoulos MD, Rigby RA, Heller GZ, Voudoris V, De Bastiani F. *Flexible Regression and Smoothing: Using GAMLSS in R*. Taylor & Francis Group; 2017.
24. Wood CL, Lane LC, Cheetham T. Puberty: normal physiology (brief overview). *Best Pract Res Clin Endocrinol Metab*. 2019;33(3):101265.
25. Grumbach MM, Styne DM. Puberty: ontogeny, neuroendocrinology, physiology, and disorders. In: Melmed S, Polonsky K, Larsen P, Kronenberg H, eds. *Williams Textbook of Endocrinology*. Elsevier; 2012:1054–1201.
26. Sakamoto H, Saito K, Oohta M, Inoue K, Ogawa Y, Yoshida H. Testicular volume measurement: comparison of ultrasonography, orchidometry, and water displacement. *Urology*. 2007;69(1):152-157.
27. Lotti F, Frizza F, Balercia G, et al. The European Academy of Andrology (EAA) ultrasound study on healthy, fertile men: an overview on male genital tract ultrasound reference ranges. *Andrology*. 2022;10(suppl 2):118-132.
28. Sørensen K, Aksglaede L, Petersen JH, Juul A. Recent changes in pubertal timing in healthy Danish boys: associations with body mass index. *J Clin Endocrinol Metab*. 2010;95(1):263-270.
29. Herman-Giddens ME, Steffes J, Harris D, et al. Secondary sexual characteristics in boys: data from the pediatric research in office settings network. *Pediatrics*. 2012;130(5):e1058-e1068.
30. Agüero G, Berner E. Concordance of testicular measurement in male adolescents with three methods of orchidometry. *Arch Argent Pediatr*. 2021;119(4):251-258.
31. van Brakel J, Kranse R, de Muinck Keizer-Schrama SM, et al. Fertility potential in men with a history of congenital undescended testes: a long-term follow-up study. *Andrology*. 2013;1(1):100-108.
32. Wikström AM, Raivio T, Hadziselimovic F, Wikström S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. *J Clin Endocrinol Metab*. 2004;89(5):2263-2270.
33. WHO Multicentre Growth Reference Study Group. WHO child growth standards based on length/height, weight and age. *Acta Paediatr Suppl*. 2006;450:76-85.
34. Saari A, Sankilampi U, Hannila ML, Kiviniemi V, Kesseli K, Dunkel L. New Finnish growth references for children and adolescents aged 0 to 20 years: length/height-for-age, weight-for-length/height, and body mass index-for-age. *Ann Med*. 2011;43(3):235-248.
35. Albertsson-Wikland K, Niklasson A, Gelander L, et al. Swedish references for weight, weight-for-height and body mass index: the GrowUp 1990 Gothenburg study. *Acta Paediatr*. 2021;110(2):537-548.
36. Bonafide CP, Brady PW, Keren R, Conway PH, Marsolo K, Daymont C. Development of heart and respiratory rate percentile curves for hospitalized children. *Pediatrics*. 2013;131(4):e1150-e1157.

37. Carlsen E, Andersen AG, Buchreitz L, et al. Inter-observer variation in the results of the clinical andrological examination including estimation of testicular size. *Int J Androl*. 2000;23(4):248-253.
38. Oehme NHB, Roelants M, Bruserud IS, et al. Ultrasound-based measurements of testicular volume in 6- to 16-year-old boys—intra- and interobserver agreement and comparison with Prader orchidometry. *Pediatr Radiol*. 2018;48(12):1771-1778.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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