Ultrasonographically measured testicular volumes in 0- to 6-year-old boys

E.A.M. Kuijper1,4, J. van Kooten1, J.I.M.L. Verbeke2, M. van Rooijen3 and C.B. Lambalk1

1Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Vrije Universiteit Medical Center (VUmc), 1007 MB Amsterdam, The Netherlands; 2Department of Radiology, Vrije Universiteit Medical Center (VUmc), 1007 MB Amsterdam, The Netherlands; 3Infant Welfare Center, GG&GD Amsterdam, Buitenveldert, Amsterdam, The Netherlands
4Correspondence address. Tel: +31-20-4440070; Fax: +31-20-4440045; E-mail: e.kuijper@vumc.nl

BACKGROUND: Aside from converted data from orchidometer measurements, there are no referential values for testicular volume ultrasound measurements in children available. Therefore, the aim of this study was to obtain ultrasonographically measured normative data for testicular volumes in 0- to 6-year-old boys. METHODS: A total of 344 boys from different ethnic backgrounds were studied. For the ultrasound measurements, an Aloka SSD-900 was used with a 7.5 mHz linear transducer. Testicular volume was calculated using the formula: length × width × height × (π/6). RESULTS: No differences were found either between the various ethnic groups or between the left and right testicle. Mean testicular volume was compared between the different age categories. Mean testicular volume increases significantly in the first 5 months from 0.27 to 0.44 cm³ after which the volume decreases to 0.31 cm³ at ~9 months. During the following years, testicular volume remains stable. CONCLUSIONS: This study provides normal values for ultrasonographically measured testicular volumes in 0- to 6-year-old boys. Ultrasound is a valid method to measure small pre-pubertal testicles as it is able to detect minor changes in volume in relation to established physiological changes in the first year of life.

Keywords: testis; volume; ultrasound

Introduction

The assessment of testicular volume has been extensively studied in recent years. In adult males, testicular volume is measured in relation to spermatogenic activity, whereas in paediatrics testicular volume measurement is mainly of importance in assessing the onset of puberty or pubertal development. It is sometimes used to evaluate testicular abnormalities such as torsio, cryptorchidism or varicocele (Fuse et al., 1990; McAlister and Sisler, 1990; Bree and Hoang, 1996). Testicular volume is related to various reproductive endocrine parameters. Therefore, it is useful to reliably measure testicular volumes in an easy and patient friendly manner. The orchidometer is widely used for this purpose in the clinical practice. The ruler is used for measuring testicular volume in adults as well, but has not been evaluated in pre-pubertal boys (Taskinen et al., 1996). A linear relation between orchidometer and ultrasound measurements has been found for testicular volumes over 4 cc (Behre et al., 1989; Taskinen et al., 1996). Overall, ultrasonography provides more accurate volumes than those obtained by orchidometer especially in small testicles (Rivkees et al., 1987; Behre et al., 1989; Ariturk and Ozates, 1993; Taskinen et al., 1996; Shiraishi et al., 2005).

Remarkably, our patient routine ultrasonographic measurements for testicular volumes in young boys showed great discrepancies with available referential value charts (Philips Medical Center, van Rijn R.R., van Robben S.G.F.). These referential data for ultrasound measurements consist of transformed orchidometer measurements (Cassorla et al., 1981; Kleinteich, 1989). With regard to ultrasonographically measured testis volumes in young boys, one paper was published on boys with cryptorchid testis (Kollin et al., 2006) and one compared testicle volumes between Danish and Finnish boys (Main et al., 2006). However, so far, no ultrasound measured referential values are available to measure testicular volumes in young boys. Therefore, the aim of this study was to obtain referential values for ultrasonographically measured testicular volumes in 0- to 6-year-old boys.

Patients and methods

Patients

A total of 344 healthy boys ranging in age from 1 to 69 months were studied. Parents of the boys were asked to have their sons testicular volumes measured by means of ultrasound during their routine visit to the welfare center. Participation rate was...
92%; three welfare centers were involved in recruitment. All boys were lying down in the supine position on the examination table with their legs spread. Boys with genital or testicular abnormalities, endocrine disorders, twins and pre-term born boys were excluded from the study population. The study was approved by the Medical Ethical committee of the Vrije Universiteit Medical Center.

In adult males, a difference in testicular volume at autopsy between different ethnic groups has been observed (Diamond, 1986). Whether this difference is already present in young boys is not clear. Therefore, we divided our subjects into groups not only by age but also by ethnic background (Caucasian, Mediterranean, African and Asian). For all four ethnic groups, we measured eight different age categories (1–2, 3–4, 5–6, 7–8, 9–10, 11–12, 13–36 and 37–70 months).

**Study procedure**

For the ultrasound measurements, a portable Aloka SSD-900 was used with a 7.5 mHz linear transducer. Sagittally, we measured length (cm) and width (cm) and transversally we measured height (cm). The sagittal diameter is characterized by the mediastinum which is identified as an echogenic line running from the superior to the inferior pole of the testis. In the sagittal diameter, the epidydimus is project separated from the testis. The epidydimus is not included in the volume measurement. Testicular volume (cm³) was calculated using the formula: length × width × height × (π/6) (Behre et al., 1989; Diamond et al., 2000; Oyen, 2002), which is the mathematical formula to measure the volume of an ellipsoid. We assumed that the testis is not a perfect ellipse, therefore width and height were measured individually, sagittally and transversally.

Baseline characteristics of the subjects were documented.

**Statistics**

A pilot study revealed that to reliably estimate the mean testicular volume, a minimum of 12 measurements per group was needed (unpublished data). Nevertheless, an aim of 30 measurements per group was considered reasonable in this study. Two investigators carried out the volume measurements. Inter-observer variation was calculated using the following equation: (measurement observer 1 − measurement observer 2)/mean of both measurements. For the intra-observer variation both measurements were done by the same investigator.

The intra-observer variation for the testicular volume was 9% (left testicle: length 5%, width 6% and height 7%, for the right testicle: length 5%, width 8% and height 11%) and the inter-observer variation was 15% (left testicle: length 8%, width 12% and height 11% and the right testicle: length 13%, width 9% and height 11%).

**Measurements**

Measurements for width and height in both testicles were significantly different in all age categories as well as for the total group (paired samples t-test for all measurements P = 0.000). The mean width and height per age category as well as the width/height ratio for the left and the right testicle are shown in Fig. 1. Whereas height showed a little increase, width showed remarkable fluctuations. Consequently, the ratio between these two measurements fluctuated sharply too, indicating large changes in testicular shape occurring over time.

**Table I.** This shows the group characteristics.

<table>
<thead>
<tr>
<th>Group characteristics</th>
<th>N = 344</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean birthweight</td>
<td>3425 gr. (± 522)</td>
</tr>
<tr>
<td>Weeks gestation</td>
<td></td>
</tr>
<tr>
<td>38–42</td>
<td>94%</td>
</tr>
<tr>
<td>&gt;42</td>
<td>6%</td>
</tr>
<tr>
<td>Breast feeding</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>84%</td>
</tr>
<tr>
<td>No</td>
<td>16%</td>
</tr>
<tr>
<td>Conception</td>
<td></td>
</tr>
<tr>
<td>Natural</td>
<td>98.9%</td>
</tr>
<tr>
<td>Induced</td>
<td>1.1% (0.4% IUI/0.7% ICSI)</td>
</tr>
<tr>
<td>Ethnical background</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>40.4%</td>
</tr>
<tr>
<td>Mediterranean</td>
<td>9%</td>
</tr>
<tr>
<td>African</td>
<td>41%</td>
</tr>
<tr>
<td>Asian</td>
<td>9.6%</td>
</tr>
</tbody>
</table>

N, number of subjects.

The group characteristics are demonstrated in Table I.

**Observer variation**

Observer variations were determined prior to the start of the study in 43 boys of random age groups. The equation used for the inter-observer variation was ((measurement observer 1 − measurement observer 2)/mean of both measurements) × 100%; for the intra-observer variation both measurements were done by the same investigator.

The intra-observer variation for the testicular volume was 9% (left testicle: length 5%, width 6% and height 7%, for the right testicle: length 5%, width 8% and height 11%) and the inter-observer variation was 15% (left testicle: length 8%, width 12% and height 11% and the right testicle: length 13%, width 9% and height 11%).

**Results**

Two boys were excluded from the study because of hypospadias and three twin pairs were excluded.

![Figure 1](image-url): The mean width and height per age category (months) as well as the width/height ratios for the left and the right testicle
**Testicular volume**

The interim analysis (ANOVA) showed no difference in left ($P = 0.950$), right ($P = 0.682$) or mean ($P = 0.841$) testicular volumes between the different ethnic groups. Linear regression analysis showed no influence of ethnicity on testicular volume either ($P = 0.670$ and $95\%$ CI-0.009–0.014). When divided into age categories, again no significant differences were found between the ethnic groups for left, right or mean testicular volumes. Therefore, data from boys originating from different ethnic groups were pooled and the study was determined.

No significant differences between the left and right testicle were found for any of the age categories (1–2 months $P = 0.208$, 3–4 months $P = 0.194$, 5–6 months $P = 0.246$, 7–8 months $P = 0.255$, 9–10 months $P = 0.295$, 11–13 months $P = 0.303$, 13–36 months $P = 0.227$ and 37–96 months $P = 0.519$ (paired samples $t$-test)). Therefore, the mean testicular volume is used as a unit of analysis per child in all the following analyses.

Birthweight and current weight were tested in a linear regression model for possible confounding effects on testicular volume. For both birthweight ($P = 0.741$) and current weight ($P = 0.792$), no significant effects were found.

**Referential values**

The mean testicular volume ($\pm 1$ or 2 SD) per age category is shown in Fig. 2.

Testicular volume increased in the first 5 months from $0.27\text{ cm}^3 (\pm 0.02)$ to $0.44\text{ cm}^3 (\pm 0.03)$ thereafter the volume decreased to $0.31\text{ cm}^3 (\pm 0.02)$ at ~9 months of age. During the following years, testicular volume remained relatively constant until the age of six.

Mean testicular volumes per month were compared using ANOVA analysis. Post hoc Bonferroni results showed a significant difference in testicular volumes between the age of 1 and 3 months ($P = 0.023$), 1 and 4 months ($P = 0.008$), 1 and 5 months ($P = 0.000$), 5 and 9 months ($P = 0.005$) and between 5 and 11 months ($P = 0.017$).

Results for the total population and for the Caucasian and African boys separately in the first year of life are shown in Fig. 3. This demonstrated the close resemblance in absolute figures and the identical shapes of the curves, each showing a double hub.

**Discussion**

This study provides ultrasonographically measured testicular volume values for 0- to 6-year-old boys, that could be used as a reference. We found the most striking changes in testicular volume to occur in the first year of life. From birth to 5 months of age, the testicular volume rises to a maximum of $0.44\text{ cm}^3 (\pm 0.03)$. After this, the volume declines again and reaches its minimum around 9 months, to remain approximately the same size till the age of six. Testicle size measurements might be important in case of, for example, a cryptorchid testis. When using the orchidometer or the ruler to measure a pre-pubertal testis, two problems arise. First, the smallest bead in the Prader orchidometer is 1 cm$^3$, while the ultrasound measured volumes in the first years of life do not exceed $0.44\text{ cm}^3$ second, the orchidometer and the ruler are known to overestimate testicular volume as they measure not only the testis but also the epidydimus as well as the skin (Rivkees et al., 1987; Fuse et al., 1990; Ariturk and Ozates, 1993; Taskinen et al., 1996). Especially in small testicles,

![Figure 2](image-url)

Figure 2: The mean testicular volume per age category (a) and the mean $\pm 1$ or 2 SD (b)

![Figure 3](image-url)

Figure 3: The mean testicular volume per month in the first year of life, for the total population and the Caucasian and African populations separately.

Numbers are given in the table.
overestimation of the volume is an important problem as the epididymus is relatively large compared to the total testicle volume.

In early infancy, testicular tissue consists mainly of Sertoli cells. Testicular volume in this period inclines as the seminiferous cord length increases resulting from proliferation of Sertoli cells (Muller and skakkebaek, 1983; Cortes et al., 1987; Rey et al., 1993; Chemes, 2001; Rey, 2003; Grumbach, 2005). The observed significant rise in testicular volume coincides with the so called ‘mini-puberty’ which describes a peak in gonadotropic hormones around 3 to 4 months of age (Andersson et al., 1998; Grumbach, 2005; Hadziselimovic et al., 2005). The hypothalamic–pituitary–gonadal axis is already capable of mature functioning, gonadotropic levels are low owing to the inhibitory effect of placental influence (Andersson et al., 1998; Winter, 1982; Grumbach, 2005). After delivery, this negative feedback from the placenta vanishes resulting in a rapid rise in serum gonadotropin levels. Not only LH and FSH, but also inhibin B and testosterone, show a peak at 3 months. Testosterone and LH return to a minimum again at 6–9 months. It takes slightly longer for FSH and inhibin B to reach the low pre-pubertal levels (Winter et al., 1975, 1976; Andersson et al., 1998; Byrd et al., 1998; Crofton et al., 2002; Chada et al., 2003; Setchell et al., 2003). Hormonal stimulation by activation of the hypothalamic–pituitary–testicular axis remains quiescent until puberty. This lack in hormonal stimulation is probably the cause of the decline in testicular volume.

Our results show that ultrasound measurement is able to detect such a small biologically relevant change in testicular volume, which indicates that this is a highly valuable and accurate method to measure the size of pre-pubertal testicles.

Visual inspection of the data during the first year gives the impression of two instead of one distinct hub. Statistically, we could not distinguish between the two, however remarkably this was present in each of the four ethnic groups, which could indicate some physiological relevance. We have no explanation, but future studies in which ultrasound testicular measurements combined with reproductive endocrine data at the time of volume measurement during the first year of life are likely to be informative in this matter.

In adult males, most of the testicular mass is composed of germ cells. Because each Sertoli cell can support the development of a limited number of germ cells, the size of the testis and its spermatogenic activity is dependent on the number of Sertoli cells present (Chemes, 2001; Rey, 2003; Sharpe et al., 2003; Grumbach, 2005; Sharpe, 2006). Ethnic background is known to influence testicular volume in adult males (Diamond, 1986). Several studies report on ethnic background and infant testicular volume, however none have compared testicle size between various ethnic groups (Beres et al., 1989; Chin et al., 1998; Matsuo et al., 2000). Our study does not show testicular size differences between pre-pubertal boys originating from various ethnic backgrounds. Apparently, a possible ethnic influence on testis size in adult males is not expressed before the age of six and probably develops during puberty. Nevertheless, recently Main et al. (2006) reported that Finnish boys, at 3 months of age, have larger testes and higher inhibin B and FSH levels compared to Danish boys.

Interpreting the differences found between the Finnish and Danish boys in light of our present findings, it is possibly the result of environmental influences instead of some genetic predisposition (Ku et al., 2002; Main et al., 2006).

Furthermore, Main et al. have measured testicular volumes in one plane. The length and width were measured and width and height were considered identical. If the testis would be a perfect ellipsoid this would have been adequate, but we showed a significant difference between width and height for each age category, indicating that the testis is not a perfect ellipsoid. As boys age, the testicle significantly changes shape, as indicated by the strong fluctuations of the width/height ratio. Therefore, the testicular volume will be over or underestimated when measured in only one plane.

In conclusion, this study provides normal values for ultrasonographically measured testicular volumes in 0- to 6-year-old boys. Ultrasound is a valid method to measure small pre-pubertal testicles, as it is able to detect minor changes in volume in relation to established physiological changes in the first year of life. The testis appears to respond to the gonadotropic surge in the neonatal period by a rise in volume which peaks at 5 months of age. Furthermore, as boys age, their testicles change shape. No differences in testicular volume between boys from different ethnic backgrounds were found.

References
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