Establishing reference values for age-related spermatogonial quantity in prepubertal human testes: a systematic review and meta-analysis

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Objective: To collect published data on spermatogonial quantity in the testes of healthy children and calculate the reference values of spermatogonial quantities throughout prepuberty.
Design: Systematic literature search in PubMed and EMBASE focusing on the number of spermatogonia per transverse tubular cross section (S/T) and spermatogonial density per cubic centimeter (cm^3) of testicular volume (S/V) throughout prepuberty.
Setting: None.
Patient(s): None.
Intervention(s): None.
Main Outcome Measure(s): Polynomial meta-regression analyses of S/T and S/V of healthy boys from the ages of 0 to 14 years.
Result(s): We found six papers describing original quantitative data on S/T and S/V of healthy boys (total n = 334 and 62, respectively) that were suitable for meta-analysis. Polynomial meta-regression analyses of S/T and S/V demonstrated a clear pattern of spermatogonial quantity throughout prepubertal life. This consisted of a decline during the first 3 years of life, a gradual increase until the ages of 6 to 7 years, a plateau until the age of 11 years, and a sharp incline reaching pubertal numbers at 13 to 14 years of age. The association between S/T and S/V allowed us to perform S/T to S/V extrapolation, creating reference S/V (rS/V) values throughout prepubertal life from a cohort of 372 boys.
Conclusion(s): Spermatogonial quantity varies during testicular development toward puberty. The values found in this study may serve as a baseline clinical reference to study the impact of diseases and adverse effects of gonadotoxic treatments on spermatogonial quantity in prepubertal testes. Spermatogonial quantity reference values may also help to evaluate the quality of testicular biopsy samples acquired for fertility preservation of prepubertal boys. (Fertil Steril® 2016;106:1652–7. Copyright ©2016 The Authors. Published by Elsevier Inc. on behalf of the American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).)
Key Words: Healthy boys, prepuberty, spermatogonial density, spermatogonial quantity, testicular development

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Spermatogonia are male germ cells located at the basal membrane of seminiferous tubules that give rise to functional spermatozoa. These early germ cells are present form birth onward, and spermatozoa are produced after puberty. The number of spermatogonia in prepubertal testes is influenced by the rate of proliferation, apoptosis, and differentiation into more advanced germ cells (1–3) as well as the growth rate of Sertoli and peritubular cells that determines the tubular length and total volume of the testis (1, 4–6). These physiologic processes can be disturbed by Klinefelter syndrome, cryptorchidism, genetic or endocrine disorders, and medical interventions like chemotherapy or irradiation and lead to partial or complete depletion of

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spermatogonia including spermatogonial stem cells during childhood (7–13).

To assess the effects of these conditions and interventions on spermatogonial counts, reference values of spermatogonial quantity throughout prepubertal life of healthy boys need to be established. To date, only a few small cohort studies have reported data on spermatogonial count per age group throughout prepuberty. Although some studies did not describe any age-related changes in spermatogonial numbers, other studies did. Therefore, we pooled data on spermatogonial quantity in human prepubertal testes by a systematic literature search and polynomial meta-regression analyses to estimate spermatogonial density throughout childhood and provide baseline clinical reference values.

MATERIALS AND METHODS

Literature Search Strategy

We used PubMed and EMBASE electronic databases to search for articles on spermatogonial number in testes of healthy prepubertal boys (final update on April 8, 2015). In PubMed, we used queries for relevant keywords and medical subject headings (MeSH) to generate three subsets of references, where the first comprised "seminiferous tubules/anatomy and histology" AND "germ cells" OR "spermatogonia" AND "germ cells/cytology" OR "cell count" OR "sperm count," the second of "apoptosis" AND "spermatozoa" OR "germ cells" OR "spermatogonia," and the third of "cell proliferation" OR "proliferation" OR "cell division" AND "spermatozoa" OR "germ cells" OR "spermatogonia," limiting all outputs by "species: humans," "sex: male," and "age: child from birth to 18 years." Similarly, we searched for terms "testicular activity" OR "testis development" OR "ontogeny" OR "prepuberty" AND "germ cell" OR "spermatogonia" OR "spermatocyte" AND "proliferation" OR "apoptosis" in EMBASE using filters "human," "male," "child." We used review papers and original research reports from this search to trace references of relevant primary data missing from the electronic search.

Study Selection and Data Extraction

We screened abstracts of the electronic search results to select developmental and quantitative reports on spermatogonia (comprising gonocytes, type A spermatogonia, and type B spermatogonia) in healthy prepubertal boys, including cases where data were reported for a control group, and excluded reports describing only spermatogonial counts of boys with (testicular) tumors, cryptorchidism, varicocele, or other health problems that might influence spermatogenesis. We summarized reported prepubertal spermatogonial cell counts per seminiferous tubular cross section (S/T) and spermatogonial numerical density per testicular tissue volume of 1 cm^3 (S/V) calculated using a stereometric counting grid by extracting data from quantitative studies. To estimate the common trend of S/T and S/V as a function of age, we pooled data from studies that specified a cohort size (n) and a range or standard deviation (SD) per each age group of their results and performed polynomial meta-regression analyses. We excluded reports that did not specify the method of spermatogonial counting or that used correction factors to adjust germ cell counts for shrinkage, tubular diameter, or tubular shape. The study selection and the data extraction strategy are summarized in the PRISMA pipeline (Fig. 1) as described previously elsewhere (14).

Statistical Analysis

We used either smoothed fractional polynomial or least square fractional polynomial without smoothing together with a random effects model as appropriate to perform the meta-regression analysis (95% confidence interval [CI]) (15). To measure the heterogeneity between values reported in the studies, we performed I^2 statistics for both S/T and S/V (16). To build reference S/V (rS/V) values, we extrapolated S/T to S/V values by using correction factors 11 (for age group 0 to 4 years) and 16 (for age group 4 to 14 years). This strategy was chosen based on the previously described constant volume density of the tubular compartment and constant testicular volume in the respective age groups, as well as individual values within each of these age groups and the described S/T to S/V correlation (6, 17). As the polynomial meta-regression data represent an estimate of the number of spermatogonia throughout prepubertal development, we considered further statistical analyses between various ages within this developmental period to be inaccurate, so we presented these as a trend. Finally, to establish reference values for age-related spermatogonial quantity in prepuberty, we extracted regression fit and 95% CI boundary values from the S/T meta-analysis, as well as boundary values for the rS/V polynomial regression. The data analysis was performed using Stata/IC 14.0 (2015; StataCorp).

RESULTS

From a total of 141 abstracts, we screened 129 full, relevant, original studies and reviews with an additional 13 articles located from references (Fig. 1). After applying the inclusion criteria, 32 full-text articles were processed. We found nine studies describing original quantitative data (Supplemental Table 1, available online), six of which satisfied the inclusion criteria and were used for S/T (5, 17–20) and S/V (6, 17) polynomial meta-regression analyses, and three (21-23) that did not satisfy the inclusion criteria. In all the selected studies, the tissue processing methodologies included histology for S/T and stereology using counting grid for S/V assessment without correction for tissue shrinking.

Changes in Spermatogonia per Tubular Cross Section (S/T) during Prepuberty

In the five studies describing S/T of healthy prepubertal boys, we identified three patterns as a function of age (Supplemental Fig. 1, available online). [1] The first pattern showed a decline in S/T numbers during the first 2 to 4 years of life followed by a gradual increase toward puberty. [2] The second pattern described S/T as a plateau from the ages of 3 to 12 years, with a sharp increase during puberty. [3] The third pattern depicted a decrease in numbers during the first 3 years