0021-972X/06/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 91(7):2732–2737

doi: 10.1210/jc.2005-2443

Larger Testes and Higher Inhibin B Levels in Finnish than in Danish Newborn Boys

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Context: Recent studies showed that male reproductive health problems, such as cryptorchidism, hypospadias, testicular cancer, and low sperm quality, are more prevalent in Denmark than in Finland.

Objectives: We hypothesized that, if fetal testicular dysgenesis contributed to these observations, differences in gonadal development and the hypothalamus-pituitary-testis axis would already be detectable perinatally. Thus, we investigated healthy newborn boys in both countries.

Design: This was a prospective, longitudinal population-based study.

Setting: Two primary obstetric centers were included at the University Hospitals of Copenhagen, Denmark, and Turku, Finland.

Participants: The participants of the study included 633 Danish and 1044 Finnish boys, born at term with appropriate weight for gestational age.

Interventions: Ultrasound determination of testis size at 0, 3, and 18 months and blood sampling (n = 727) at 3 months were analyzed.

Main Outcome Measures: Testicular volume and reproductive hormones were measured.

Results: Testis volume was significantly higher at all ages in Finnish than in Danish boys (medians, 98 *vs.* 95, 185 *vs.* 119, and 188 *vs.* 136 mm^3, respectively; $P < 0.00001$). Testis growth from birth to 3 months was larger in Finnish than in Danish boys (mean, 75 *vs.* 26 mm^3; $P < 0.0001$). Serum hormone levels were higher in Finnish than Danish boys for inhibin B (median, 456 *vs.* 385 pg/ml; $P < 0.0001$), FSH (1.33 *vs.* 1.21 IU/liter; $P < 0.036$), and SHBG (143 *vs.* 136 nmol/liter; $P < 0.022$). Inhibin B was significantly positively correlated to testicular volume ($r = 0.25$; $P < 0.006$).

Conclusions: The larger testes and higher inhibin B levels most likely represent a bigger volume of seminiferous tubules in Finnish compared with Danish boys. Although this phenomenon may be attributable to a genetic difference between the two countries, it may also reflect environmental factors influencing testicular development. **(*J Clin Endocrinol Metab* 91: 2732–2737, 2006)**

RECENTLY, SIGNIFICANT GEOGRAPHIC and temporal differences in male reproductive health, including testicular cancer, undescended testes, spermatogenic disorders, and hypospadias, have been reported (1–7). Some of these findings caused controversies (8, 9). However, remarkable geographical differences have been confirmed in many studies (10–13). The etiologies of these trends are largely unknown, but it has been hypothesized that some of the problems in adulthood may be attributable to testicular dysgenesis in fetal life (14, 15). A well-described difference in male reproductive health problems exists between Denmark and Finland. Denmark has a 4-fold higher incidence of testicular cancer, and semen quality of Danish men from the general population is significantly poorer than that of Finnish men. During the past years, Nordic researchers have used this gradient to study possible genetic and environmental influences on male reproductive disorders (16–19).

To test the hypothesis that fetal testicular dysgenesis may contribute to the remarkable differences in reproductive health between the two Nordic countries, our research groups in Copenhagen and Turku performed a joint study of 1677 newborn boys from the two countries with systematic assessment of testicular volume at birth and during the first 18 months of life, as well as analysis of pituitary-gonadal hormones from blood samples taken at 3 months of age.

Subjects and Methods

Study participants and design

We performed a large joint longitudinal birth cohort study of newborn boys between 1997 and 2001 at the University Hospitals in Copenhagen, Denmark (Rigshospitalet and Hvidovre Hospital), and Turku University Central Hospital, in Turku, Finland (3). Pregnant women, belonging to the primary referral area of the hospital, were recruited consecutively and prospectively in the first trimester of pregnancy to avoid selection bias. Women referred to the tertiary obstetric unit for complicated pregnancy were excluded. Only families of Danish and Finnish origin, respectively, were included to minimize genetic and geographical variation within each country. Participation rates were 22% in Denmark and 24% in Finland (3). In this study, only those boys were included who were born at term (gestational age, 37–42 wk), with appropriate birth weight for gestational age (20, 21) and with no signs of cryptorchidism, hypospadias, or foreskin malformations on any examination. The total number was 633 in Denmark and 1044 in Finland (including nine twins in Denmark and four in Finland; $P < 0.019$).

First Published Online April 4, 2006
Abbreviation: CV, Coefficient of variation.

Information regarding maternal medical history, smoking, parity, and parturition was obtained from hospital records. A total of 26.5% of the Danish and 24.1% of the Finnish mothers smoked during pregnancy ($P < 0.191$). A total of 1.4% of the Danish and 6.9% of the Finnish mothers had diabetes or impaired glucose tolerance ($P < 0.0001$), and 62% were primipara in Denmark and 52% in Finland ($P < 0.0001$).

Standard orchidometer measurements were considered to be too inaccurate and insensitive to distinguish testis size in this age group. Thus, testicular volume was measured by ultrasound in both centers (Aloka SSD-500; Aloka, Tokyo, Japan) with a linear 7.5 MHz transducer (8 mm footprint, model UST-5521U-7,5) to measure length (millimeters) and width (millimeters). Three workshops were held for all investigators from both countries to train and standardize the ultrasound technique.

Each measurement (length and width) was performed three times, and the mean was calculated. Testicular volume (cubic millimeters) was calculated by the equation of an ellipsoid: length (mm) × width (mm^2) × $\pi/6$ (22) for each testis. Mean testicular volume was calculated as the mean of right and left side.

In Denmark, all boys were examined at birth and 3 and 18 months of age. In Finland, the boys were seen at birth and at 3 months. For funding reasons, only a subgroup was hereafter seen at 18 months of age. This subgroup consisted of: 1) every 10th of healthy boys from the cohort (by date of birth), and 2) matched controls for boys with cryptorchidism [matched by parity, maternal diabetes, and smoking (yes/no); date of birth, ±14 d; gestational age, ±7 d].

Quality controls were performed by plotting all ultrasound measurements of each observer for each age group to check for trends over time attributable to training effect. A total of 9.7% (n = 419) of all ultrasound size measurements were omitted from the final analysis because of the following criteria: erroneous inclusion of epididymis, extreme outlier (above 2 SD) in one of three length or width measurements, or bad technical quality judged by the printout. One Finnish observer showed a considerable trend toward smaller measurements over the 18 months, hereafter measurements were stabilized. From this observer, all measurements from the first 18 months (n = 178) were omitted from the analysis. A total of 3607 Danish and 4701 Finnish unilateral ultrasound examinations of left and right testis were included in the analysis (n = 1797 and 2339 bilateral measurements, respectively). This material includes observations of one observer who examined infants in both countries, first in Finland (n = 19 and 245) and then in Denmark (n = 94 and 111) at 0 and 3 months, respectively. The intraobserver variation (±2 SD) was determined from all three individual measurements of testis length and width of all children in the study. Intraobserver variation was for testis length ±1.5 mm [coefficient of variation (CV), 16%] and for width ±1.3 mm (CV, 25%). The interobserver variation was determined in 44 boys, in whom ultrasounds were performed blinded by two consecutive observers during the same clinical examination, covering all combinations of investigators. Each observer performed three length and three width measurements of each testis. The interobserver variation for length measurements was ±2.3 mm (CV, 24%) and for width was ±1.4 mm (CV, 27%).

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 (Baby scale model; Solotop Oy, Helsinki, Finland) to the nearest 0.005 kg. Body surface area (square meters) was calculated by the equation of Haycock (23): weight $(kg)^{0.5378}$ × height $(cm)^{0.3964}$ × 0.024265.

Nonfasting peripheral venous blood samples were taken from an antecubital vein at a median of 1200 h (0900 to 1600 h) in Denmark and a median of 1400 h (1200 to 1630 h) in Finland. In this study, only blood samples taken exactly between 2.5 and 3.5 months (76–106 postnatal days) of age were included (n = 409 Danish and 318 Finnish samples). Danish boys were a median of 90 d old (77–105 d), and Finnish boys were 93 d old (84–102 d). Samples were separated by centrifugation and stored at −20 C. All blood samples were analyzed as duplicates and blinded for the technician at one laboratory (Rigshospitalet, Copenhagen, Denmark). Each run contained blood samples from both countries to minimize any effect of interassay variation.

Ethics

The study was performed according to the Helsinki II declaration. The parents gave their written informed consent. Approval was obtained from the local Ethics Committees in Denmark and Finland, respectively, and for a joint database from the Danish Registry Agency.

Hormonal assays

FSH and LH were measured by two-sided time-resolved immunofluorometric assays (Delfia; Wallac, Turku, Finland) with detection limits of 0.05 IU/liter and intraassay and interassay CVs of less than 3% and less than 5%, respectively.

Serum estradiol was measured by a RIA (Pantex, Santa Monica, CA). The intraassay CV was less than 8%, and the interassay CV was less than 13%. The detection limit was 18 pmol/liter.

Serum inhibin B was measured by a specific ELISA (Oxford Bio-Innovation, Oxford, UK) with a detection limit of 20 pg/ml. Intraassay and interassay CVs were less than 13% and less than 19%. The cross-reactivity for inhibin A in this specific assay was 0.5% (24, 25). The ratio between inhibin B and FSH was calculated by simple division [inhibin B (pg/ml)/FSH (IU/liter)].

Testosterone was measured by a solid-phase RIA (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA) without previous extraction. The detection limit was 0.23 nmol/liter, and the intraassay and interassay CVs were less than 10%.

SHBG was determined by a time-resolved fluoroimmunoassay (Delfia; Wallac) with a detection limit of 0.23 nmol/liter and intraassay as well as interassay CVs of less than 5%. Free testosterone (picomoles/liter) was calculated using the equation suggested by Vermeulen (26). We applied an average albumin concentration for age of 40 g/liter (27), because albumin was not measured individually in our samples.

Statistical analysis

All descriptive statistics are given as medians and 2.5/97.5 percentiles unless otherwise stated. Serum hormone levels below the limit of detection for the given assay were set to the limit of detection for statistical analysis.

Country differences in population characteristics, serum hormone concentrations, and testicular volumes were tested by Mann-Whitney (two groups) or Kruskall-Wallis (more than two groups) tests.

Multiple linear regression analysis was used to test factors of significance for testicular volume at 0, 3, and 18 months of age. Log-transformed data for testicular volume (in cubic millimeters and cubic millimeters per square meter) were used as the dependent variable, and country of origin (Finland, 1; Denmark, 2), parity, diabetes (1, yes; 2, no), maternal age and height, and weight for gestational age were used as independent covariates.

Partial correlation analyses between FSH and inhibin B were done after logarithmic transformation to achieve normal distribution and by controlling for country of origin. Correlations between hormone values, population characteristics, or anthropometric measurements and testis volume were tested by partial correlation controlling for observer. All statistical analyses were performed using SPSS software (version 13.0; SPSS, Chicago, IL).

Results

The description of the two study populations is given in Table 1. Maternal age and height were significantly higher in Denmark than in Finland. There were small, but statistically significant, differences in gestational age and weight for gestational age between the two countries. Infant length was greater in Danish than Finnish boys at birth and, to a very small extent, also at 3 months of age.

There was a highly significant difference in mean testicular volume determined by ultrasound between the countries, with Finnish boys having larger testicles at all ages than Danish boys (Fig. 1). Mean testicular volume was at birth 98 mm^3 (66–288 mm^3) in Finland and 95 mm^3 (46–207 mm^3) in Denmark ($P < 0.012$). At 3 months, testicular volume had increased to 185 mm^3 (118–299 mm^3) in Finland and 119 mm^3 (55–251 mm^3) in Denmark ($P < 0.0001$). At 18 months, tes-

TABLE 1. Study population characteristics

	Denmark (n = 633)	Finland (n = 1044)	*P* value
Maternal age (yr)	30.6 (23.1–39.3)	29.2 (20.6–39.4)	0.0001
Maternal height (cm)	169 (158–181)	166 (155–178)	0.0001
Gestational age (d)	282 (262–294)	280 (262–293)	0.002
Weight for gestational age (%)	−0.81 (−18.8/18.4)	0.49 (−18.5/19.0)	0.014
Birth weight (kg)	3.62 (2.75–4.46)	3.65 (2.84–4.42)	0.390
Weight, 3 months (kg)	6.51 (5.19–7.90)	6.56 (5.25–7.94)	0.372
ΔWeight, 0–3 months (kg/3 months)	3.0 (1.8–4.7)	2.9 (1.8–4.2)	0.03
Weight, 18 months (kg)	11.7 (9.75–14.31)	11.9 (9.75–14.83)	0.025
ΔWeight, 3–18 months (kg/15 months)	5.0 (3.4–7.1)	5.2 (3.7–7.3)	0.001
Birth length (cm)	53 (48–57)	51 (47–55)	0.0001
Length, 3 months (cm)	62.2 (58.6–67.1)	62.0 (58.0–65.5)	0.0001
ΔHeight, 0–3 months (cm/3 months)	10.3 (6.3–15.4)	10.7 (7.6–13.8)	0.0001
Length, 18 months (cm)	83.7 (78.6–89.6)	84.0 (79.0–89.5)	0.076
ΔHeight, 3–18 months (cm/15 months)	20.5 (16.5–24.3)	21.9 (18.2–25.8)	0.0001

Data represent median (2.5 and 97.5 percentiles). The *P* value describes country differences (Mann-Whitney test).

ticular volume was 188 mm^3 (125–243 mm^3) in Finland and 136 mm^3 (64–246 mm^3) in Denmark ($P < 0.0001$). The subgroup of children seen by the same observer in Finland and Denmark, respectively, showed the same findings [at birth, 151 mm^3 (96–304 mm^3) *vs.* 76 mm^3 (46–114 mm^3), $P < 0.0001$; at 3 months, 200 mm^3 (124–365 mm^3) *vs.* 109 mm^3 (56–173 mm^3), $P < 0.0001$]. In the multivariate analysis, the country of origin ($r = -0.205$; $P < 0.0001$) and weight for gestational age ($r = 0.109$; $P < 0.008$) had a significant influence on testicular volume at birth, at 3 months ($r = -0.613$, $P < 0.0001$ and $r = 0.073$, $P < 0.001$, respectively), and at 18 months ($r = -0.473$, $P < 0.0001$ and $r = 0.07$, $P < 0.046$, respectively). At 3 months, maternal diabetes had an additional significant influence on testicular volume ($r = 0.041$; $P < 0.048$). There were no significant correlations of testicular volume at birth to gestational age, parity, maternal diabetes, or smoking. There was no significant correlation between testis volume at any age and parity.

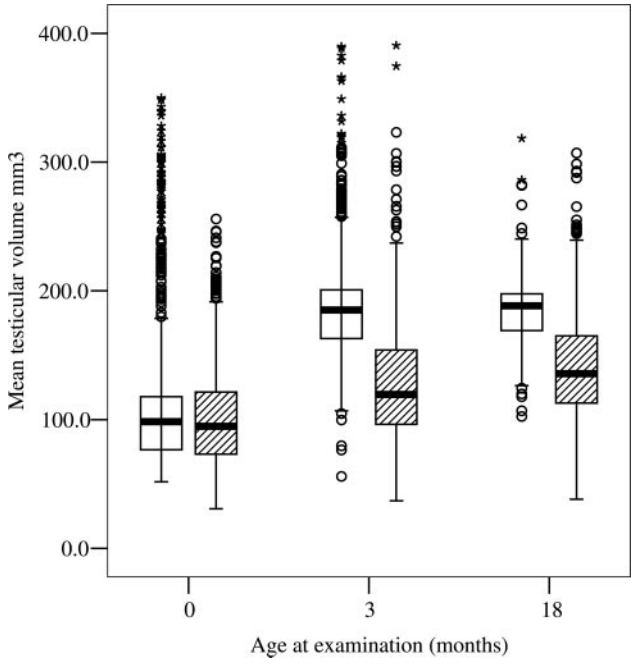

FIG. 1. Mean testicular volume (cubic millimeters) determined by ultrasound at approximately 0 months (n = 625 and 1032), 3 months (n = 619 and 1018), and 18 months (n = 553 and 289) of age in healthy Danish and Finnish infant boys, respectively. The box plots represent median and quartiles, outliers (○), and extremes (*). Finland is shown as *white boxes*, and Denmark is shown as *striped boxes*.

Testis growth from birth to 3 months of age was significantly higher in Finland than in Denmark (mean ± SEM, 75 ± 2 *vs.* 26 ± 2 mm^3; $P < 0.0001$). The growth in testicular volume was significant between all ages in Denmark ($P < 0.0001$ for all age combinations) and between examination at birth and either 3 or 18 months in Finland ($P < 0.0001$). Between 3 and 18 months of age, Danish boys showed continued testis growth (15 ± 3 mm^3), whereas in the Finnish boys, testis size remained unchanged (−0.4 ± 3 mm^3; $P < 0.0001$).

Body surface area (square meters) was significantly correlated to testis volume at all ages ($r = 0.062–0.163$; $P < 0.0001–0.012$). Body length (centimeters) and weight (kilograms) were not correlated to testis volume at birth, but at 3 and 18 months ($r = 0.099–0.150$; $P < 0.001$) they were. Mean testicular volume adjusted for body surface area (cubic millimeters per square meter) was significantly smaller in Danish than in Finnish boys at all ages (Table 2). In Denmark, testicular volume adjusted for body surface showed a significant decrease from 0 to 3 and from 3 to 18 months of age ($P < 0.0001$). In Finland, there was a significant increase in testicular volume adjusted for body surface from 0 to 3 months ($P < 0.0001$) and a significant decrease from 3 to 18 months ($P < 0.0001$). In both countries, testicular volume adjusted for body surface was significantly smaller at 18 months than at birth ($P < 0.0001$). In the multivariate analysis, the country of origin ($r = -0.206$; $P < 0.0001$) and weight for gestational age ($r = -0.07$; $P < 0.007$) had a significant influence on testicular volume per square meter at birth. At 3 months, country of origin ($r = -0.614$; $P < 0.0001$), maternal diabetes ($r = 0.041$; $P < 0.051$), and maternal height

TABLE 2. Testis volume adjusted for body surface (cubic millimeters per square meter) at birth (n = 624 and 1032) and at 3 months (n = 616 and 1004) and 18 months (n = 547 and 284) in healthy Danish and Finnish boys, respectively

	Denmark	Finland	*P* value
At birth	407 (197–918)	425 (268–1257)	0.003
3 months	349 (161–746)	533 (351–872)	0.0001
18 months	256 (124–464)	343 (233–479)	0.0001

Data represent median (2.5 and 97.5 percentiles). *P* values for country differences were obtained by Mann-Whitney test.

($r = -0.051$; $P < 0.018$) had a significant influence on testicular volume per square meter. At 18 months, only the country of origin was significant ($r = -0.438$; $P < 0.0001$).

We found significant country differences for inhibin B, FSH, and SHBG with higher serum concentrations in Finnish than in Danish boys (Table 3), whereas the inhibin B/FSH ratio did not differ. LH, FSH, inhibin B, and SHBG were measurable in all samples. Serum estradiol was at or below detection limit for the assay in 221 of 522 samples (42%), 47% in Denmark and 32% in Finland ($P < 0.003$). Serum testosterone was undetectable in three of 720 boys (0.4%), two Danish and one Finnish.

Mean testicular volume at 3 months of age and serum concentration of inhibin B were positively correlated (Fig. 2), the partial correlation coefficient being $r = 0.25$ after controlling for observer ($P < 0.0001$). There were no significant correlations between any other reproductive hormones and testicular volume. Inhibin B was negatively correlated to FSH ($r = -0.348$; $P < 0.0001$) and positively to testosterone ($r = 0.125$; $P < 0.005$), free testosterone ($r = 0.125$; $P < 0.005$), and LH ($r = 0.162$; $P < 0.0001$).

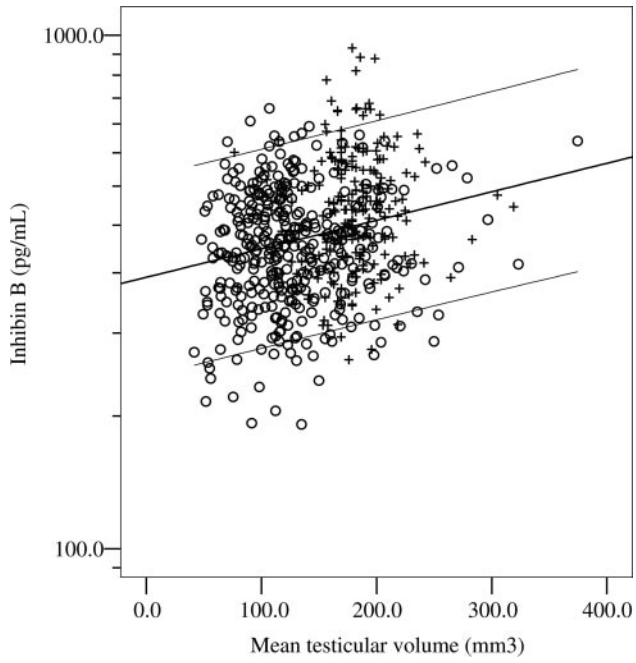

FIG. 2. Correlation between testicular volume (ultrasound measurement in cubic millimeters) and serum inhibin B levels (picograms per milliliter) at 3 months of age (partial correlation coefficient, $r = 0.25$; $P = 0.0001$). +, Finland; ○, Denmark. The y-axis is logarithmic.

Discussion

Our closely coordinated study of 633 Danish and 1044 Finnish boys demonstrated that Danish boys had smaller testes as neonates and throughout infancy, even if data were adjusted for general body growth. The difference was small at birth but increased to approximately 30% at 3 and 18 months, caused by a smaller postnatal testicular growth in the Danish boys. Our findings most likely reflect a difference in the volume of seminiferous tubules because Danish boys had 16% lower levels of inhibin B and 9% lower levels of pituitary FSH than Finnish boys. Inhibin B is a marker of Sertoli cell function and closely related to the size of the Sertoli cell pool (27, 28). Thus, these two outcome measurements, *i.e.* testis size and inhibin B, corroborate each other.

The prevalence of undescended testes and hypospadias is significantly more frequent in Danish newborn boys than in Finnish (3, 4, 7). Furthermore, there is convincing evidence that, not only is testis cancer several times more frequent in Denmark than in Finland (10), but semen quality is poorer (5, 6, 11, 29). The reasons for this remarkable geographic difference are not known. The synchrony between male reproductive diseases (14) and the present findings would be in line with a hypothesis of a more frequent occurrence of impaired perinatal testicular development among Danish males. Whereas genital malformations are true reproductive disorders, it is yet unknown whether the smaller size and growth of the testes in otherwise healthy Danish infants may have biological consequences in adulthood. However, in young Danish men, testicular volume was significantly smaller than in Finnish men, and testicular size was correlated to semen quality (5). Boys with small testis size may be "programmed" for a higher risk of reduced semen quality and testicular cancer, which most likely is of fetal origin (30, 31). In rats, reduction of Sertoli cell number in newborn pups led to a decrease in the Sertoli cell population and spermatid production in adult animals (32, 33). However, follow-up studies are needed to show whether differences in early postnatal gonadal size and function are also predictive of adult reproductive health in humans. The two populations differed slightly in maternal and infant characteristics. However, infant body size did not explain our findings, because testis size adjusted for body surface area showed significantly smaller testis in Danish as opposed to Finnish boys.

TABLE 3. Serum levels of reproductive hormones and growth factors in 720 healthy 2.5- to 3.5-month-old Danish and Finnish boys born at term (gestational age, 37–42 wk) with appropriate birth weight for gestational age

	Denmark (n = 409)	Finland (n = 318)	Country difference, *P* value
FSH (IU/liter)	1.21 (0.41–3.04)	1.33 (0.49–3.08)	0.036
Inhibin B (pg/ml)	385 (231–634)	456 (272–762)	0.0001
Inhibin B/FSH ratio	329 (88–1286)	355 (111–1354)	0.124
LH (IU/liter)	1.77 (0.55–4.15)	1.77 (0.59–4.06)	0.873
Testosterone (nmol/liter)	3.29 (0.78–7.58)	3.31 (0.65–7.89)	0.755
Free testosterone (pmol/liter) (26)	21.5 (5.7–44.1)	20.9 (4.1–44.9)	0.639
SHBG (nmol/liter)	136 (66–266)	143 (77–268)	0.022
Estradiol (pmol/liter)	20 (18–44)	23 (18–45)	0.007

Data represent median (2.5 and 97.5 percentiles). *P* values for country differences were obtained by Mann-Whitney test.

Maternal factors such as parity, smoking, height, and age showed no significant influence on infant testis volume in a multivariate model. Gestational diabetes had a very weak positive influence on testis size only at 3 months, and, given the low prevalence of gestational diabetes (6.9% in Finland, 1.4% in Denmark), this does not explain the observed difference in testis size. We found an intriguing positive association between weight for gestational age within normal limits and testis size, which was strongest at birth and diminished toward 18 months. This may indicate a general interaction between fetal gonadal development and intrauterine body growth.

From birth to 3 months of age, the gonads are stimulated by gonadotropins and testicular volume increases, as shown by measurement with orchidometer (34) or in autopsy material (35, 36). FSH may also be important for testicular growth in infancy, as indicated by a case report of an 8-month-old boy with hypogonadotropic hypogonadism, in whom treatment with recombinant gonadotropins induced testicular growth (37). The postnatal FSH in the Finnish boys was significantly higher than in the Danish, which may explain the lack of additional increase in testicular volume from 3 to 18 months when the gonadotropin drive diminishes. Histologically, testicular growth represents an increase in the length of seminiferous tubules, total germ cell number (35), and Sertoli cell number (38, 39), and decreased apoptosis of Sertoli cells (40, 41). The observed negative feedback between FSH and inhibin B was similar to that described for pubertal boys and adults (42–44). Inhibin B levels correlate with testis size (45, 46) and reflect Sertoli cell function. The absolute number of Sertoli cells can vary more than 10-fold between adults, giving rise to large interindividual variation in inhibin B levels, as also seen here (28, 45). Thus, the difference in absolute size as well as testis growth between Danish and Finnish boys may reflect a true difference in the Sertoli cell pool, with a higher set point of the hypothalamus-pituitary-gonadal axis in the Finnish boys.

The fact that the same geographic difference in testicular size was found by one observer who examined children in both countries seems to rule out interobserver variation between the countries as a likely cause. We excluded 178 ultrasounds from one observer because of an obvious training effect over time. However, this did not change the conclusions, because the population differences in fact were larger if these data were included. We did not find significant population differences in total and free testosterone or LH. This may be caused by the relatively brief postnatal peak of these hormones, which can be missed by taking only one sample per child.

In conclusion, the smaller size and postnatal growth of the testis and lower levels of the Sertoli cell-specific hormone inhibin B in the Danish newborns most likely reflect a lower volume of seminiferous tubules in the Danish boys. These results are noteworthy in relation to the high birth prevalence of genital abnormalities, one of the world's highest incidences of testicular cancer, and widespread occurrence of poor semen quality among young men in Denmark. Our findings in this representative cohort of healthy newborn from both countries may be attributable to genetic differences between Danish and Finnish males. However, they may also reflect an adverse effect of environmental factors on perinatal testicular development.

Acknowledgments

We thank research nurse Helle Kelkeland as well as technicians Kirsten Jørgensen, Ulla Højelse, and Ole Nielsen for their skilled assistance, and we are especially grateful to all families who participated in this study. We also thank the staff of the Obstetric and Neonatal Units of both University Hospitals for their warm support and practical help in conducting the study.

Received November 8, 2005. Accepted March 24, 2006.

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This work was supported by European Commission Grants QLK4-CT1999-01422, QLK-2001-00269, and QLK4-2002-0063, Danish Medical Research Council Grants 9700833 and 9700909, the Sven Andersens and Velux Foundations, the Novo Nordisk Foundation, the Sigrid Jusélius Foundation, The Turku University Central Hospital, and the Academy of Finland. The sponsors had no part in study design, data collection, analysis, interpretation, or writing of the manuscript and decision for submission. The article does not represent the opinion of the European Community, who is not responsible for any use that might be made of data appearing therein.

References

1. **Adami H-O, Bergström R, Möhner M, Zatonski W, Storm H, Ekbom A, Tretli S, Teppo L, Ziegler H, Rahu M, Gurevicius R, Stengrevics A** 1994 Testicular cancer in nine Northern European countries. Int J Cancer 59:33–38
2. **Clemmesen J** 1981 Testis cancer incidence: suggestion of a world pattern. Int J Androl Suppl 4:111–122
3. **Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto A-M, Schmidt IM, Chellakooty M, Damgaard IN, Mau C, Reunanen M, Skakkebaek NE, Toppari J** 2004 Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363:1264–1269
4. **Boisen KA, Chellakooty M, Schmidt I, Kai CM, Damgaard IN, Suomi AM, Toppari J, Skakkebaek NE, Main KM** 2005 Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at 3 months of age. J Clin Endocrinol Metab 90:4041–4046
5. **Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen A-G, Andersson AM, Haugen TB, Horte A, Jensen TK, Magnus O, Petersen JH, Vierula M, Toppari J, Skakkebaek NE** 2002 East-West gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod 17:2199–2208
6. **Carlsen E, Giwercman A, Keiding N, Skakkebæk NE** 1995 Declining semen quality and increasing incidence of testicular cancer: is there a common cause? Environ Health Perspect 103:137–139
7. **Virtanen HE, Kaleva M, Haavisto A-M, Schmidt IM, Chellakooty M, Main KM, Skakkebaek NE, Toppari J** 2001 The birth rate of hypospadias in the Turku area in Finland. APMIS 109:96–100
8. **Jouannet P, Wang C, Eustache F, Jensen TK, Auger J** 2001 Semen quality and male reproductive health: the controversy about human sperm concentration decline. APMIS 109:48–61
9. **Paulsen CA, Berman NG, Wang C** 1996 Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. Fertil Steril 65:1015–1020
10. **Purdue MP, Devesa SS, Sigurdson AJ, McGlynn KA** 2005 International patterns and trends in testis cancer incidence. Int J Cancer 115:822–827
11. **Jensen TK, Vierula M, Hjollund NHI, Saaranen M, Scheike T, Saarikoski S, Suominen J, Keiski A, Toppari J, Skakkebaek NE** 2000 Semen quality among Danish and Finnish men attempting to conceive. Eur J Endocrinol 142:47–52
12. **Jørgensen N, Andersen A-G, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EH, Horte A, Jensen TK, Jouannet P, Keiding N, Vierula M, Toppari J, Skakkebaek NE** 2001 Regional differences in semen quality in Europe. Hum Reprod 16:1012–1019
13. **Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW; Study For Future Families Research Group** 2003 Geographic differences in semen quality of fertile U.S. males. Environ Health Perspect 111:414–420
14. **Skakkebæk NE, Rajpert-De Meyts E, Main KM** 2001 Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972–978
15. **Virtanen HE, Rajpert-De Meyts E, Main KM, Skakkebaek NE, Toppari J** 2005

Testicular dysgenesis syndrome and the development and occurrence of male reproductive disorders. Toxicol Appl Pharmacol 207(Suppl 2):501–505
16. **Shen H, Main KM, Kaleva M, Virtanen HE, Haavisto A-M, Skakkebæk NE, Toppari J, Schramm KW** 2005 Prenatal organochlorine pesticides in placentas from Finland: exposure of male infants born during 1997–2001. Placenta 26: 512–514
17. **Jensen TK, Jørgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus O, Matulevicius V, Nermoen I, Vierula M, Keiding N, Toppari J, Skakkebaek NE** 2004 Association of *in utero* exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. Am J Epidemiol 159:49–58
18. **Jensen TK, Andersson A-M, Jørgensen N, Andersen A-G, Carlsen E, Petersen JH, Skakkebaek NE** 2004 Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril 82:863–870
19. **Jensen TK, Giwercman A, Carlsen E, Scheike T, Skakkebæk NE** 1996 Semen quality among members of organic food associations in Zealand, Denmark. Lancet 347:1844
20. **Marsál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B** 1996 Intrauterine growth curves based on ultrasonically estimated foetal weights. Acta Paediatr 85:843–848
21. **Larsen T, Petersen S, Greisen G, Larsen JF** 1990 Normal fetal growth evaluated by longitudinal ultrasound examinations. Early Hum Dev 24:37–45
22. **Lenz S, Giwercman A, Elsborg A, Cohr K-H, Jelnes JE** 1993 Ultrasonic testicular texture and size in 444 men from the general population: correlation to semen quality. Eur Urol 24:231–238
23. **Haycock GB, Schwartz GJ, Wisotsky DH** 1978 Geomatric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. J Pediatr 93:62–66
24. **Groome NP, Illingworth PJ, O'Brien M, Cooke I, Ganesan TS, Baird DT, McNeilly AS** 1994 Detection of dimeric inhibin throughout the human menstrual cycle by two-site enzyme immunoassay. Clin Endocrinol (Oxf) 40:717–723
25. **Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, McNeilly AS** 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 81:1401–1405
26. **Vermeulen A, Verdonck l, Kaufman JM** 1999 A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 84:3666–3672
27. **Stapleton RD, Plotnick L** 1993 Diagnostic and therapeutic information, Part II. In: Johnson KB, ed. The John Hopkins Hospital: The Harriet Lane Handbook. 13th ed. St. Louis: Mosby; 89–96
28. **Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ** 1996 Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. J Clin Endocrinol Metab 81:3341–3345
29. **Andersson A-M, Jørgensen N, Larsen LF, Rajpert-De Meyts E, Skakkebæk NE** 2004 Impaired Leydig cell function in infertile men: a study of 357 idiopathic infertile men and 318 proven fertile controls. J Clin Endocrinol Metab 89:3161–3167
30. **Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson A-M, Krarup T, Keiding N, Skakkebaek NE** 2000 High frequency of sub-optimal semen quality in an unselected population of young men. Hum Reprod 15:366–372
31. **Høi-Hansen CE, Rajpert-De Meyts E, Carlsen E, Almstrup K, Leffers H, Skakkebæk NE** 2005 Fast-track article: a subfertile patient diagnosed with testicular carcinoma in situ by immunocytological staining for AP-2γ in semen samples: case report. Hum Reprod 20:579–582
32. **Almstrup K, Høi-Hansen CE, Wirkner U, Blake J, Schwager C, Ansorge W, Nielsen JE, Skakkebaek NE, Rajpert-De Meyts E, Leffers H** 2004 Embryonic stem cell-like features of testicular carcinoma in situ revealed by genome-wide gene expression profiling. Cancer Res 64:4736–4743
33. **Orth JM, Gunsalus GL, Lamperti AA** 1988 Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. Endocrinology 122:787–794
34. **Cassorla FG, Golden SM, Johnsonbaugh RE, Heroman WM, Loriaux DL, Sherins RJ** 1981 Testicular volume during early infancy. J Pediatr 99:742–743
35. **Müller J, Skakkebæk NE** 1983 Quantification of germ cells and seminiferous tubules by stereological examination of testicles from 50 boys who suffered from sudden death. Int J Androl 6:143–156
36. **Müller J, Skakkebæk NE** 1984 Fluctuations in the number of germ cells during the late foetal and early postnatal periods in boys. Acta Endocrinol 105:271–274
37. **Main KM, Schmidt IM, Toppari J, Skakkebæk NE** 2002 Early postnatal treatment of hypogonadotropic hypogonadism with recombinant human FSH and LH. Eur J Endocrinol 146:75–79
38. **Cortes D, Müller J, Skakkebæk NE** 1987 Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. Int J Androl 10:589–596
39. **Rey RA, Campo SM, Bedecarrás P, Nagle CA, Chemes HE** 1993 Is infancy a quiescent period of testicular development? Histological, morphometric, and functional study of the seminiferous tubules of the cebus monkey from birth to the end of puberty. J Clin Endocrinol Metab 76:1325–1331
40. **Berensztein E, Saraco N, Belgorosky A, Rivarola MA** 2000 Secretion of inhibin B by human prepubertal testicular cells in culture. Eur J Endocrinol 142:481–485
41. **Sharpe RM, Fraser HM, Brougham MFH, McKinnell C, Dorris KD, Kelnar CJH, Wallace WHB, Walker M** 2003 Role of the neonatal period of pituitary-testicular activity in germ cell proliferation and differentiation in the primate testis. Hum Reprod 18:2110–2117
42. **Andersson A-M, Juul A, Petersen JH, Müller J, Groome NP, Skakkebæk NE** 1997 Serum inhibin B in healthy pubertal and adolescent boys: relation to age, stage of puberty, and follicle-stimulating hormone, luteinizing hormone, testosterone and estradiol levels. J Clin Endocrinol Metab 82:3976–3982
43. **Byrd W, Bennett MJ, Carr BR, Dong Y, Wians F, Rainey W** 1998 Regulation of biologically active dimeric inhibin A and B from infancy to adulthood in the male. J Clin Endocrinol Metab 83:2849–2854
44. **Crofton PM, Evans AE, Groome NP, Taylor MR, Holland CV, Kelnar CJ** 2002 Inhibin B in boys from birth to adulthood: relationship with age, pubertal stage, FSH and testosterone. Clin Endocrinol (Oxf) 56:215–221
45. **Crofton PM, Illingworth PJ, Groome NP, Stirling HF, Swanston I, Gow S, Wu FC, McNeilly A, Kelnar CJ** 1997 Changes in dimeric inhibin A and B during normal early puberty in boys and girls. Clin Endocrinol (Oxf) 46:109–114
46. **Manasco PK, Umbach DM, Muly SM, Godwin DC, Negro-Vilar A, Culler MD, Underwood LE** 1995 Ontogeny of gonadotropin, testosterone, and inhibin secretion in normal boys through puberty based on overnight serial sampling. J Clin Endocrinol Metab 80:2046–2052

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