Increased frequency of reproductive health problems among fathers of boys with hypospadias

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BACKGROUND: Some studies have suggested an association between paternal subfertility and hypospadias among their sons, although the association has not been systematically investigated. We therefore compared male reproductive health among a group of fathers of boys with hypospadias and a group of fathers to normal children.

METHODS: A total of 64 fathers of boys with hypospadias participated; 349 partners of pregnant women served as a control group. All men delivered a semen sample, had a blood sample drawn, underwent a physical examination and completed a questionnaire. RESULTS: Fathers of boys with hypospadias had a significantly lower median sperm concentration (54.1 × 10⁶/ml) (P = 0.004) and total sperm count (222.0 × 10⁶) (P = 0.009) than the controls (81.2 and 326.0 × 10⁶/ml). In addition, the fathers of boys with hypospadias more often reported to have had disorders in the urogenital system (hypospadias, cryptorchidism and testicular cancer) (11/64) (P < 0.001) than the control group (16/349). No significant differences in waiting time to pregnancy was observed, however, 15% of fathers to boys with hypospadias had received fertility treatment. CONCLUSIONS: Fathers of boys with hypospadias not only have an increased frequency of hypospadias, but also decreased semen quality. Most likely fathers and sons share the same susceptibility genes for reproductive dysfunction, but additional impact of environmental factors cannot be excluded.

Keywords: hypospadias; male reproductive health; semen quality

Background

The birth prevalence of hypospadias has been reported to be increasing in certain geographical regions (Paulozzi, 1999; Boisen et al., 2005), but the aetiology is in most cases unknown. Furthermore, hypospadias has been hypothesized to be a symptom in the disease complex testicular dysgenesis syndrome (TDS) which includes low sperm quality, testicular cancer, hypospadias and cryptorchidism (Skakkebæk et al., 2001), probably caused by dysfunction of Sertoli cells and/or Leydig cells in fetal life. The four conditions share risk factors which are all coupled to the intrauterine milieu, such as birth weight, premature birth and low parity (Berkowitz et al., 1995; Sharpe, 2003; Aschim et al., 2004; Pierik et al., 2004). Furthermore, each of the disorders is a risk factor for the others. Thus, testicular cancer is associated with reduced semen quality and decreased fertility even in the years before the cancer is diagnosed (Berthelsen and Skakkebæk, 1983; Skakkebæk et al., 1987; Jacobsen et al., 2000; Richiardi et al., 2004). In addition, it is well documented that cryptorchidism and other signs of maldevelopment of the reproductive system are risk factors for testicular cancer (Krabbe et al., 1979; Prener et al., 1996; Dieckmann and Pichlmeier, 2004). However, the association between hypospadias and the other disorders of the male reproductive system has been less well documented, partly because hypospadias is a rare condition, and partly because it has been underreported in registries. However, studies in selected populations of patients with testicular cancer (Prener et al., 1996), cryptorchidism (Hjertkvist et al., 1989; John Radcliffe Hospital Cryptorchidism Study Group, 1992; Akre et al., 1999) and hypospadias (Sweet et al., 1974; Bau et al., 1979; Khuri et al., 1981; Bracka, 1989; Weidner et al., 1999; Wu et al., 2002) revealed an association between hypospadias and the other reproductive disorders.

Assuming that some cases of hypospadias may be due to genetic factors which may also cause other reproductive problems, one would expect to find an increased frequency of reproductive problems including hypospadias among fathers of boys with hypospadias. In fact, some studies have suggested an association between paternal subfertility and hypospadias among the offspring (Sweet et al., 1974; Czeizel and Toth, 1990; Källén et al., 1991; Fritz and Czeizel, 1996). However, the validity of these studies is low, mainly due to methodological shortcomings, such as too small series, no reliable control groups, low participation rates or retrospectively selected populations with known fertility problems. Therefore, we
conducted a study to investigate male reproductive health and semen quality among a group of fathers of boys with hypospadias.

Materials and Methods

Fathers of boys with hypospadias were identified from a large ongoing case-control study of families with boys diagnosed or operated for hypospadias (n = 306). Boys with hypospadias were included when they were referred to the University Department of Paediatric Surgery, Rigshospitalet or the University Department of Plastic and Reconstructive Surgery, Herlev Hospital for primary operation or pre-operative evaluation. Experienced surgeons examined the boys, and the type of hypospadias and associated malformations was registered in a standardized registration form. Boys with previous operations for hypospadias were excluded. The fathers residing in the Copenhagen area were invited to participate in an additional study about their semen quality (n = 145).

An eligibility criterion for the father to participate was that he and his mother had to have been born in Denmark. Participation in the study was accepted even if the man had a past history of cryptorchidism, orchitis, epididymitis, surgery of the genital tract (including varicocelectomy), chemotherapy, radiotherapy or other diseases, which may affect reproduction. Chronic illnesses, previous treatment for infertility or subfertility, unwanted pregnancy or prolonged waiting time to pregnancy (TTP) were not exclusion criteria. A total of 145 fathers were asked whether they were interested in receiving information about this study; 118 fathers accepted and 64 fathers participated (participation rate 44%). There were no differences in age of the fathers or the boys or their type of hypospadias between the groups of participants and informed, non-participants (n = 54), but a tendency toward increased distance to the hospital and a older age of the child was found in the group of men who did not receive information about the study (n = 27; Table 1).

A total of 349 fertile men from the general population previously investigated (Jørgensen et al., 2001) served as controls (participation rate 44%). They were recruited when their partners showed up for their first antenatal visit at the hospital. The men lived in the same catchment’s area and were included under the same eligibility criteria as the fathers of boys with hypospadias with the exception that the pregnancy had to be achieved by normal sexual relations, and not as a result of any treatment for subfertility or infertility (hormonal treatment, insemination, IVF or ICSI etc.). The information for informed consent was identical in the two groups.

The inclusion period was: February 2005–March 2006 in the study of fathers of boys with hypospadias, and September 1996–October 1997 in the control group. All examinations took place at the University Department of Growth and Reproduction, Rigshospitalet, Copenhagen.

All study subjects received 60€ for their participation (including travel compensation). The study was conducted according to the Helsinki II declaration and was approved by the local ethics committee, and the Danish Data protection Agency.

Questionnaires

On the day of attendance the men returned a completed standardized questionnaire, which they had received by mail in advance. The questionnaire included information on age, health, occupational factors, waiting TTP, genital diseases such as hypospadias, cryptorchidism, testicular cancer, phimosis, inguinal hernia, epididymitis and operation for torsio testis. They also reported their smoking habits and alcohol intake during the week before the completion of the questionnaire. Smoking habits were reported as the average number of cigarettes, cigars or pipes smoked per day. Total weekly alcohol intake (number of drinks) was calculated by summarizing beer, wine and liquor intake. The questionnaire was similar in the two groups (Jørgensen et al., 2001).

Physical examination

Physical examination of each participant was performed on the day of the delivery of the semen sample. One physician (C.A.) performed all examinations in the group of fathers of boys with hypospadias whereas another physician had conducted the examination of the control group. Evaluations of testes disposition, consistency (normal or soft) and varicocele were performed with the men in standing position. A Prader orchidometer as well as ultrasound was used to determine the volume of the testis. In addition, weight and height were measured, and BMI was calculated as weight in kilograms divided by squared height in meters.

Semen samples

The participants delivered one semen sample obtained by masturbation in a room close to the semen laboratory. The period of abstinence was recorded and the semen sample was analysed according to the World Health Organization's guidelines.

Table 1: Characteristics of participating and non-participating fathers of boys with hypospadias

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Non-participating fathers of boys with hypospadias</th>
<th>Participating fathers of boys with hypospadias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Did not receive information material (n = 27)</td>
<td>Received information material (n = 54)</td>
</tr>
<tr>
<td>Age of the father (mean, years)</td>
<td>32.5</td>
<td>32.5</td>
</tr>
<tr>
<td>Age of the boy (mean, years)</td>
<td>3.9</td>
<td>4.4</td>
</tr>
<tr>
<td>The boys type of hypospadias (frequency, %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granular</td>
<td>12.0</td>
<td>14.6</td>
</tr>
<tr>
<td>Coronal</td>
<td>36.0</td>
<td>36.6</td>
</tr>
<tr>
<td>Penile</td>
<td>52.0</td>
<td>31.7</td>
</tr>
<tr>
<td>Penoscrotal</td>
<td>0.0</td>
<td>17.1</td>
</tr>
<tr>
<td>Geographic living areaa (frequency, %)</td>
<td>40.0</td>
<td>47.2</td>
</tr>
<tr>
<td>1</td>
<td>24.0</td>
<td>28.3</td>
</tr>
<tr>
<td>2</td>
<td>36.0</td>
<td>24.5</td>
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<td>3</td>
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</table>

*a118 fathers out of 145 parents of boys diagnosed or operated for hypospadias agreed to receive information on the study (non-participants = 27). b64 fathers out of the total number of 118 agreed in participating in the study (informed, non-participating fathers = 54). cNo information on the age of the father was obtained. d1: copenhagen city and county; 2: nearer Copenhagen area; 3: distant Copenhagen area.
to the World Health Organization guidelines (1999) modified in accordance with Jørgensen et al. (1997; 2001; 2002). Sperm concentration was assessed by the use of a Bürker-Türk haemocytometer. Since 1996, our laboratory has lead a quality control program for assessment of sperm concentration. For all of these years the laboratory has kept the inter-laboratory difference unchanged in comparison with two other laboratories that have also participated since the start of the program, and we have previously taken advantage of this program in other publications (Jørgensen et al., 2001, 2002; Punab et al., 2003).

Semen smears were fixed and stained by the Papanicolaou method at the time of semen delivery. The same experienced technician assessed the sperm morphology for both the cases and the controls according to strict criteria (WHO, 1999) within 21 consecutive working days. All slides were analysed randomly and without knowledge of the identity of the groups. A set of slides scored by the technician that contributed to Guzik et al. (2001), was used as the standard for purposes of quality control. On each of the 21 working days, our technician scored two of these slides and compared the results with the standard value before starting the actual assessment. If there was any disagreement, the slides were reassigned until agreement. The semen smears of the fertile men in the control group have previously been assessed in France as part of a multicenter study using David criteria (Jørgensen et al., 2001), and some slides were accidentally damaged during the transportation. Thus, only 226 slides were available for reassessment analysis in the control group. We do, however, believe that they are a random sample of the 349 original slides. The slides from the control group were stained at the time the men were examined (1996–1997) and stored in darkness to avoid fading.

**Blood samples**

A blood sample was withdrawn from a cubital vein of each participant, then centrifuged, and the serum was separated and frozen. All samples were analysed for hormones in the same laboratory. Serum levels of FSH, LH and sex hormone-binding globulin (SHBG) were determined using time-resolved immunofluorometric assays (Delfia, Wallac, Turku, Finland). The intra-assay variation was <1.3, <1.7 and <5.0% for the FSH, LH and SHBG assay, respectively. The inter-assay variation was 6.8, 4.4 and 4.8%, respectively. Testosterone levels were determined using a time-resolved fluoroimmunoassay (Delfia). The intra- and inter-assay variation was 1.7, and 13.2%, respectively. Estradiol (E2) was determined in the control group by radioimmunoassay (Pantex, Santa Monica, CA, USA) and in the group of fathers of boys with hypospadias by a time-resolved fluoroimmunoassay (Delfia). The two subtractive 4.5

The free testosterone (FT) concentration was calculated using the equation of Vermeulen et al. (1999), and a fixed albumin concentration of 43 g/l. Because changes in the concentration of albumin only have minute effects on the ratio between total and FT, it is justifiable to use a fixed mean albumin concentration when individual albumin measurements are not available, provided there is no reason to suspect significantly abnormal albumin levels. Additionally, the free androgen index [FAI = (T/SHBG)*100] was calculated.

**Statistical analysis**

Outcome variables were semen volume, sperm concentration, total sperm count, the percentage of motile and morphologically normal spermatozoa, reproductive hormones and testicular size.

Semen and hormone parameters among fathers of boys with hypospadias were compared with the control group. Normally distributed outcome variables were entered directly as continuous variables in a linear multiple regression analysis, whereas sperm concentration and total sperm count were transformed by use of the natural logarithm to obtain normality.

The distribution of related variables to semen and/or hormone parameters and lifestyle was compared in the two groups. Differences in age, height, weight, BMI, testicular sizes, self-reported previous genital diseases, previous pregnancies and TTP from the questionnaire and genital diseases found at the clinical examination between the fathers of boys with hypospadias and the control group were tested by chi-squared test. Potential confounders were then entered in multiple linear regression analysis and excluded stepwise if they were not statistically significant at the 10% level. The age of the man, birth cohort, season of year, smoking, alcohol, BMI, previous genital diseases and duration of abstinence were evaluated as possible confounders for the semen parameters. For percentages of motile sperm, the duration from ejaculation to assessment of motility was evaluated as an additional confounder. Increasing duration of abstinence had an increasing effect on semen volume, sperm concentration and total sperm count up to ~96 h (P < 0.001) for all three parameters, where after no further effect of a longer abstinence period could be observed. Furthermore, duration of >30 min from ejaculation to assessment had a decreasing effect on sperm motility. In the final model, we therefore adjusted for period of abstinence ~96 h in the analysis of semen volume, sperm concentration and total sperm count, and adjusted for the duration from ejaculation to assessment in the analyses of the sperm motility. The linear multiple regression analysis as described above was repeated in a subgroup of fathers without the TDS related disorders: cryptorchidism, hypospadias and testicular cancer, that were transformed into one variable for each outcome (present or not present).

The results are presented with 95% confidence intervals (95% CI). The fit of the regression models was evaluated by testing the residuals for normality and by inspecting the residual plots. For testosterone and FT, hour of the day of blood sampling and BMI was evaluated as possible confounders. We did not find any effect of the hour of day of blood sampling or of BMI on the hormone values, and the Mann–Whitney test was used to compare the hormonal levels between the groups. An additional r-test was performed and gave similar results to those from the Mann–Whitney analysis.

**Results**

A total of 64 fathers of boys with hypospadias participated. Three of the fathers had been vasectomized and were excluded in the analysis of the semen sample. A total of 349 fertile men served as controls. Ten (15.6%) of the fathers of boys with hypospadias reported to have received treatment for subfertility: six couples had been treated with insemination and of these, three couples specifically reported to having received additional hormonal treatment; one couple had IVF; two couples were treated with ICSI and one couple reported hysterosalpingography as fertility treatment. None of the fathers in the control group had received fertility treatment to obtain the present pregnancy since this was an exclusion criteria in...
the study, however, eight (2.3%) reported to having received fertility treatment in a previous pregnancy.

Table 2 shows the frequencies of self-reported genital diseases and diseases in the reproductive organs found at the physical examination; 12.5% of the fathers of the boys with hypospadias had hypospadias themselves (0% among controls) and more had been operated for phimosis or inguinal hernia, or treated for cryptorchidism or testicular cancer. Overall, 17.5% of the fathers of boys with hypospadias had one or more of the following conditions: cryptorchidism, hypospadias or testicular cancer, in contrast to 4.6% among controls (Table 2).

The unadjusted semen parameters are summarized in Table 3. Based on the unadjusted values, 23% of the fathers of boys with hypospadias had a semen concentration <20 × 10^6/ml (WHO lower normality level) in contrast to 12.4% (P = 0.022) in the control group. At the clinical examination, the fathers of boys with hypospadias had significantly smaller mean size of testis as measured by orchidometer (21.5 ml; P = 0.023) and by ultrasound (14.8 ml; P < 0.001) than controls (23.0 and 17.5 ml, respectively).

After adjustment for duration of ejaculation of abstinence up to ~96 h, fathers of boys with hypospadias had a significantly lower median sperm concentration (54.1 × 10^6/ml; P = 0.004) and total sperm counts (222.0 × 10^6; P = 0.009) than the controls (81.2 and 326.0 × 10^6). No statistical significant differences in semen volume, or percentages of morphologically normal or motile spermatozoa were found between the groups (Table 3).

We did separate analyses of fathers who had no history of cryptorchidism, testicular cancer or hypospadias (Table 3), and even among these ‘healthy’ fathers of boys with hypospadias, the sperm concentration and total sperm count were lower than in controls.

In addition, we did separate analyses of the data from the fathers of the boys with hypospadias, who had never received fertility treatment (n = 51), and after adjustment for duration of ejaculation of abstinence up to ~96 h, the fathers in this group still had a lower median sperm concentration (52.7 × 10^6/ml) (P = 0.004) and a lower total sperm count (226.8 × 10^6/ml) (P = 0.02) than the controls. Interestingly, 15.6% of the fathers of boys with hypospadias had received fertility treatment, which is higher than the general Danish population [6.2% in Denmark (Nyboe and Erb, 2006)].

Waiting TTP was significantly longer in the group of fathers of the boys with hypospadias (P < 0.001) than in the control group (Table 2). The proportion who had conceived in <3, 6 and 12 months were 61, 72 and 86%, respectively, among the couples of boys with hypospadias compared with 70, 85 and 93% among the controls. Additionally, the percentage of fathers who reported to previously having been responsible for a pregnancy was lower in the group of fathers of boys with hypospadias 34.4% compared with 53.6% in the control group (P < 0.001). However, when the TTP among fathers of the boys with hypospadias who had never received fertility treatment (n = 54) were compared with controls, no difference in mean and median TTP was found.

The results of the serum reproductive hormones are summarized in Table 4. The fathers of boys with hypospadias had lower levels of testosterone (P = 0.008) and FT (P < 0.001) and higher levels of E2 (P = 0.001) than did controls. The levels of E2 were not corrected according to the assay used, which means that the real differences between the groups may have been even greater (see discussion below). In addition, we found a decreased inhibin B/FSH ratio among the fathers of boys compared with controls (P = 0.06), which could be
interpreted as an indicator of impaired spermatogenesis (Petersen, 2003).

Subgroup analysis: fathers with hypospadias

Eight (12.5%) fathers of boys with hypospadias had hypospadias themselves as stated above. One of these fathers had previously been operated for testicular cancer, three had been treated for cryptorchidism, and two had been operated for inguinal hernia. No association between type of hypospadias in the boys and the unadjusted sperm concentration and the type of hypospadias among the fathers was found (data not shown). The adjusted median sperm concentration was 22.0 × 10^6/ml (95% CI: 6.4, 75.3) among fathers with hypospadias compared with 64.2 × 10^6/ml (95% CI: 33.9, 121.8) in fathers without hypospadias. The percentage of motile cells and morphologically normal sperm cells was 65.0% (95% CI: 55.5, 74.5) (adjusted value) and 10.6% (95% CI: 6.8, 14.4), respectively, among fathers with hypospadias compared with 65.0% (95% CI: 60.5, 69.4) (adjusted value) and 9.1% (95% CI: 7.8, 10.4) in fathers without hypospadias. The fathers with hypospadias had smaller testicles (mean testicular volume measured by UL: 11.3 ml, 4.2 SD) than the fathers without hypospadias (15.2 ml, 7.05 SD) (P < 0.001). The percentage of fathers with hypospadias who had a TTP ≤ 12 months was 37.5% compared with 10.7% of the fathers without hypospadias (P = 0.140).

Discussion

We found that fathers of boys with hypospadias had a lower sperm concentration and total sperm count than the control group. No differences in other semen parameters between the groups were found. However, the fathers of boys with hypospadias more often reported having had disorders in the urogenital system (cryptorchidism, inguinal hernia, testicular cancer and phimosis).

We also found differences in reproductive hormone levels between the groups. Fathers of boys with hypospadias had higher FSH and lower inhibin B values (although the latter were not significantly) which is in line with the lower sperm concentration in this group. Additionally, the fathers of boys with hypospadias may also have a slightly reduced Leydig cell function as the testosterone levels (including calculated FT levels) were significantly reduced compared with controls, even after adjustment for BMI. The reproductive hormone levels should however be interpreted with caution since the serum samples from the two groups of men were analysed 10 years apart, and our findings could also be due to the inter-assay variation, which for testosterone was 13.2%. Furthermore, two different assays were used to determine the levels of E2. From comparison of the two methods, it was known that the assay used to measure E2 in fathers of boys with hypospadias gave on average 12% lower results than the assay used to measure the controls. Nevertheless, we found significantly higher levels in the fathers, thus the real difference between the two groups may be even larger. Previous publications have found a diurnal rhythm in serum levels of reproductive
hormones (Carlsen *et al*., 1999; Jørgensen *et al*., 2002). We therefore included hour of day of blood sampling in our initial analysis of hormones, but found no effect of this on the hormone parameters. An explanation could be that nearly all of the participants had their blood sample drawn before 10 AM.

The fathers of boys with hypospadias and the control group were not directly comparable. First, they were recruited 10 years apart. Semen quality has been reported to decline were not directly comparable. First, they were recruited 10 years apart. Semen quality has been reported to decline 10 years apart. Semen quality has been reported to decline with increasing year of birth (Auger *et al*., 1995; Irvine *et al*., 1996; Bonde *et al*., 1998). However, the majority of men in both groups belonged to the same birth cohort (1965–1969). The age of fathers of boys with hypospadias was higher than that of the control group. However, we controlled for a possible age effect, which did not change the results in semen quality.

Second, the laboratory practices did not change over the study period. However, as the men were investigated 10 years apart, an intra-laboratory variation cannot be excluded in advance. To partly overcome this problem, we have practiced high quality control standards in our laboratories and some of the same technicians have been employed over the whole period. Morphology assessment was carried out blindly and our laboratory coordinated an external quality control program for assessment of sperm concentration, which has shown a minimal intra-laboratory variation over time. These quality control procedures have previously shown their values in studies in regional differences in semen quality (Jørgensen *et al*., 2001,2002; Punab *et al*., 2003). Furthermore, fathers of boys with hypospadias and the control group answered the same questionnaire and were examined identically. We therefore believe that our results represent a true difference in reproductive health between the fathers of boys with hypospadias and the control group.

Finally, for the man in the control group to be included his child had to have been ‘conceived naturally’ and not as a result of any treatment for subfertility or infertility, whereas this was not an exclusion criteria in the group of fathers of boys with hypospadias. No difference in TTP between the fathers of boys with hypospadias who did not receive fertility treatment and the control group was found. However, a considerable number of fathers of boys with hypospadias (15.6%) had received fertility treatment. The percentage of children conceived after some form of fertility treatment is 6.2% in Denmark (Nyboe and Erb, 2006). This suggests that fathers of boys with hypospadias experience fertility problems to a higher extent than the general Danish population. In addition, the participation rates between the fathers of boys with hypospadias and controls were quite similar. Furthermore, no differences in characteristics between the participating and non-participating fathers of boys with hypospadias were found, and therefore it is most likely that the fathers of boys with hypospadias are representative of the group of men having sons with hypospadias.

Our findings in the group of fathers of boys with hypospadias are in accordance with the hypothesis that reduced parental fertility is associated with hypospadias and notably with paternal problems, although no reliable data on semen quality have previously been reported (Sweet *et al*., 1974; Kallen *et al*., 1986; Czeizel and Toth, 1990; Fritz and Czeizel, 1996; Wennerholm *et al*., 2000; Pierik *et al*., 2004; Bonduelle *et al*., 2005). However, an Hungarian study found a lower number of morphologically normal and motile spermatozoa among 25 fathers of boys with hypospadias (Fritz and Czeizel, 1996). Furthermore, our findings are also in line with a retrospective study based on medical reports on 70 fathers of boys with hypospadias, that found a higher proportion of abnormalities of the testes and scrotum in fathers of boys with hypospadias (Sweet *et al*., 1974).

A high number of fathers of boys with hypospadias received fertility treatment which is in accordance with previous studies that found an association between longer waiting TTP in the parents of boys with hypospadias (Czeizel and Toth, 1990; Pierik *et al*., 2004) and a higher frequency of infertility requiring treatment among fathers of boys with hypospadias (Fritz and Czeizel, 1996). In addition, a lower percentage of fathers of boys with hypospadias had previously been responsible for a pregnancy, despite a higher age at the time of inclusion compared with controls. Studies of an association between parity and risk of hypospadias are conflicting (Ericson *et al*., 1987; Czeizel and Toth, 1990; Källén *et al*., 1991; Weidner *et al*., 1999). An international case-control study examined 846 children with isolated hypospadias (Källén *et al*., 1991). Couples with boys with hypospadias had slightly fewer previous pregnancies and an increased number of infertility periods of at least 6 months than controls, although the difference did not

<table>
<thead>
<tr>
<th></th>
<th>Fathers of boys with hypospadias, n = 64</th>
<th>Control group, n = 349</th>
<th>Difference between the groups*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Median (5–95)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Inhibin B (pg/ml)</td>
<td>203.0 (93.8)</td>
<td>194.5 (57.5–369.5)</td>
<td>225.0 (86.9)</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.0 (4.4)</td>
<td>3.5 (1.4–14.6)</td>
<td>3.7 (2.2)</td>
</tr>
<tr>
<td>Inhibin B/FSH ratio</td>
<td>71.6 (65.4)</td>
<td>55.1 (4.3–249.3)</td>
<td>87.1 (77.8)</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>3.9 (1.6)</td>
<td>3.8 (1.6–7.2)</td>
<td>3.9 (1.6)</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>457.7 (133.6)</td>
<td>457.6 (254.2–711.2)</td>
<td>520.7 (127.7)</td>
</tr>
<tr>
<td>Testosterone (nm/l)</td>
<td>20.4 (6.9)</td>
<td>19.9 (10.1–35.7)</td>
<td>22.3 (6.9)</td>
</tr>
<tr>
<td>E2 (pmol/l)</td>
<td>75.2 (17.6)</td>
<td>71.0 (52.0–109.8)</td>
<td>67.1 (21.5)</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>32.3 (34.0)</td>
<td>30.0 (14.0–57.8)</td>
<td>31.4 (13.0)</td>
</tr>
</tbody>
</table>

reach statistical difference. Our control group of fertile men represents normal fertile men from the Danish population as they were included irrespective of their semen quality and waiting TTP. Fathers of boys with hypospadias were included up to two years after the pregnancy whereas the fathers of normal boys were included during pregnancy. Therefore, the fathers of boys with hypospadias had a longer period of recall. TTP has, however, been proven to be well recalled, even many years after the pregnancy both among men and women (Joffe et al., 1995; Jensen et al., 2005).

We found a familial aggregation of hypospadias (12.5% of the fathers had also hypospadias). Other studies have found prevalences between 3.5 and 6% (Sørensen, 1953; Sweet et al., 1974). Inguinal hernia has been linked to other TDS-like symptoms in several studies (Møller et al., 1996; Prener et al., 1996; Weidner et al., 1999). The frequency of previous inguinal hernia was surprisingly high in the group of fathers of boys with hypospadias: 14.9%, in contrast to 4.9% in the control group and another Danish study (Møller and Skakkebæk, 1996) in which the frequency was 9.3%. However, as observed by Møller et al. (1996) there is a potential for confusion of cryptorchidism and inguinal hernia when the data is obtained by questionnaire or interview, and therefore the results should be interpreted cautiously. In addition, cryptorchidism and phimosis were more common among fathers of boys with hypospadias compared with the controls and to findings in other studies, in which the prevalence of cryptorchidism observed at birth was 3–5% (Boisen et al., 2004) and the frequency of phimosis was, respectively, 0.6 (Shankar and Rickwood, 1999) and 1.7% (Oster, 1968).

In conclusion, our results indicate that the fathers of boys with hypospadias not only had an increased frequency of hypospadias, but also decreased semen quality. Most likely the father and son share the same susceptibility genes for reproductive dysfunction, although we cannot exclude environmental factors as causes, as the father and son to some extent may also share the same environment. If this is the case, our results would seem to be in line with new data showing that environmental agents may generate their effects through genetic imprinting (Klip et al., 2002; Anway et al., 2005; Brouwers et al., 2006).

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References


Nyboe AA, Erb K. Register data on assisted reproductive technology (ART) in Europe including a detailed description of ART in Denmark. *Int J Androl* 2006;29:12–16.


Skakkebæk NE, Raipert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Hum Reprod* 2001;16:972–978.

Sørensen HR. *Hypospadias. With Special Reference to Aetiology*. Munksgaard: København, 1953;1–94.


