Abnormal sperm morphology and function in the fathers of hypospadiacs

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A lower motility of spermatozoa and a higher incidence of abnormal sperm morphology were found in 25 fathers of hypospadiacs compared with those of 50 fathers who produced children who were not hypospadiacs. Subfertility of fathers may result in a higher risk of hypospadias in offspring and as subfertile males now represent a higher proportion among fathers, owing to the improved efficacy of infertility treatment, this may explain the increased occurrence of hypospadias. Our relaxed-selection hypothesis, which states that there is a redistribution in the number of children born to fertile and infertile (subfertile) couples, may account for the increasing number of other defects and cancers of male genitalia observed today and the fall in sperm counts.

Introduction

In recent years, adverse changes have been detected in male reproductive health including a decrease in sperm concentration and quality (Carlsen *et al.*, 1992; Auger *et al.*, 1995, although this was questioned by Olsen *et al.*, 1995), an increase in the incidence of testicular cancer (Osterlind, 1986; Forman and Moller, 1994) and of congenital abnormalities of the male reproductive organs such as testis retention and hypospadias. The incidence of hypospadias at birth has increased significantly over past decades in Norway (Bjerkedal and Bakkeiteig, 1975), Sweden (Källen and Winberg, 1979), the UK (Matlai and Beral, 1985) and Hungary (Czeizel *et al.*, 1986). Genetic factors have a role in the origin of hypospadias (Sorensen, 1953; Roberts and Lloyd, 1973) which corresponds to the Galtonian multifactorial-threshold model, that is, genetic predisposition is triggered by environmental factors (Czeizel *et al.*, 1979; Czeizel and Tusnády, 1984). In a previous study, the role of parental subfertility in the occurrence of hypospadias was indicated (Czeizel and Tóth, 1990). This finding prompted us to propose the relaxed-selection hypothesis. This hypothesis is based on the following facts and findings. A century ago, natural fertility resulted on average in eleven children being born to fertile couples (Toulemon, 1988). At the present time, couples with good fertility use strict birth control (the number of children per fertile couple is fewer than two in Hungary) while the efficacy of treatment of infertile and subfertile couples has improved. In Hungary, about 12–15% of couples were previously childless; this figure is now about 6–8%. Thus, there has been a redistribution in the number of children born to fertile and infertile couples: in 1920 only 4% of babies were born to couples classed as subfertile before treatment; this figure was estimated at 14% in 1980 (Czeizel *et al.*, 1986). The hypothesis was tested in a blind case-control study of semen

from the fathers of children with hypospadias and the results are presented here.

Patients and methods

Patients affected by isolated hypospadias (hypospadiac cases with other genital and extragenital defects were excluded) were ascertained from the dataset of the Hungarian Congenital Abnormality Registry, 1985–1987 (Czeizel, 1988a). The fathers of 49 hypospadiac children, living in Budapest, were invited to participate in this study. Participation was optional and 25 fathers (51%) gave their informed consent. Two men were excluded from the study because they had chronic diseases. The remaining 22 men did not wish to take part. Personal data and fertility history of the participants were obtained by personal interview. The men were asked to collect semen in an appropriate receiver using coitus interruptus at home after 3 days of sexual abstinence. In general, coitus interruptus is not an acceptable means of semen collection because it is probable that the first portion of the ejaculate, which usually contains the highest concentration of spermatozoa, will be lost. However, this was considered as the most appropriate method as masturbation was refused by several men. Semen samples were delivered to our laboratory within 1 h of collection and samples were protected from extremes of temperature (less than 20°C and more than 40°C) during transport. The period of abstinence, the date and time of collection, and the interval between collection and analysis were recorded (Table 1).

Two controls were recruited for each case father. They were selected from participants of the Optimal Family Planning Service (Czeizel, 1988b) on the basis of being almost the same age (± 2 years), residence in the same area and that they had fathered at least one healthy baby. Personal data were obtained and semen was collected by the same method as that used by the fathers of hypospadiacs.

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Bias in scoring was avoided by coding samples with scorers having no prior knowledge of which samples were from the study group and which from the control group. Evaluation of semen samples was based on three characteristics: sperm density analysis, sperm motility and sperm morphology.

Sperm density. A fixed volume of semen was delivered on to a clean glass slide using a micropipette and covered with a 22 mm × 22 mm coverslip. The wet preparation was left to stabilize for approximately one minute, and the preparation was examined at a magnification of × 600 at room temperature.

Sperm motility. The motility of 100 spermatozoa was estimated as (1) rapid and progressive, (2) slow and sluggishly progressive, (3) nonprogressive and (4) immotile. The count was repeated in a second drop of semen and the average values were calculated for each category.

Sperm morphology. An analysis of abnormalities in morphology observed after Giemsa staining (WHO, 1992) was performed on smears containing 100 spermatozoa in seminal fluid. Four categories of morphological defects were identified and the percentage occurrence of these four abnormal forms were scored. These categories were (1) defects of the head; (2) defects of the neck and midpiece; (3) tail defects; and (4) abnormalities of the cytoplasmic droplet which is usually located in the neck-midpiece region of the cell. The technique used to measure sperm concentration and grade sperm motility can provide only approximate results.

Statistical analyses

The data were analysed initially by the chi-squared test to determine normality (Kendall and Stuart, 1968). For the evaluation of semen variables the Mann-Whitney test was used (Walters, 1993) apart from the mean of sperm density when if the distribution was normal Student's *t* test was used. Chi-squared and Student's *t* test were used for the comparison of confounding factors.

Results

The demographic data of case and control fathers did not show any significant differences with the exception that the number who had completed grade at school was higher in control fathers (Table 1). Case and control fathers were healthy. The proportion of smokers was somewhat, but not significantly, higher in case fathers than in control fathers.

The technical criteria (for example, period of abstinence, the interval between collection and analysis) of semen examination did not show significant differences between fathers of hypospadiacs and the aged-matched fathers of healthy children (Table 1). Macroscopic features, such as liquefaction, appearance, volume, consistency and pH, were not significantly different between the study and control groups.

Table 1. Demographic characteristics of participants and data on semen collection from fathers of children with hypospadias (study group) and men who had healthy children (control group)

Characteristics of fathers	Study group (n = 25)	Control group (n = 50)	P value
Mean (± SD) age (yr)	28.6 ± 2.9	29.2 ± 4.1	0.51
Number (%) of children			
1	13 (52)	31 (62)	0.67
2	11 (44)	18 (36)	
3	1 (4)	1 (2)	
Occupation (%)			
Professional	9 (36)	20 (40)	0.88
Clerk	2 (8)	2 (4)	
Manual worker	12 (48)	25 (50)	
Other	2 (8)	3 (6)	
Number (mean ± SD) who completed grade at school	11.8 ± 3.3	13.4 ± 2.4	0.008
Cigarette smoker			
Yes	7 (28)	12 (24)	0.93
Former	4 (16)	9 (18)	
No	14 (56)	29 (58)	
Alcohol consumption (%)			
Abstainers	5 (20)	7 (14)	0.78
Occasional drinkers	11 (44)	25 (50)	
Regular drinkers	9 (36)	18 (36)	
Mean (± SD) time (min) from collection to analysis	53.5 ± 11.7	55.0 ± 10.5	0.56
Days of sexual abstinence (%)			
3	21 (84)	38 (76)	0.63
4	2 (8)	8 (16)	
5 or more	2 (8)	4 (8)	
Mean (± SD) age (yr) of spouse at birth of child	25.0 ± 2.91	25.6 ± 4.4	0.58

The sperm density of case and control fathers is shown (Table 2). The distribution of data was normal ($\chi^2_6 = 7.6$; $P = 0.27$). The Mann-Whitney test indicated a significantly different distribution in case fathers than in control fathers. However, the mean was not significantly lower in case fathers compared with the mean of control fathers ($t_{74} = -1.95$; $P = 0.06$). The distribution for motility characteristics was not normal ($\chi^2_8 = 218.0$; $P < 0.000$) (Table 2). The grading of motility indicated significantly lower motility of spermatozoa in the study group compared with the control group. The morphological classification of spermatozoa also showed a significant difference between the study and control samples because some categories of defects, mainly small heads, was significantly higher in the study group than in the control group (Table 3).

Of the 25 fathers of hypospadiacs studied, 6 (24%) reported past subfertility requiring medical treatment (based on the inability to achieve a planned conception within one year when their wives were considered healthy); while of the 50 control men only 3 (6%) had a previous history of subfertility ($\chi^2_1 = 5.11$; $P = 0.02$).

Table 2. Comparison of sperm density and motility in semen from fathers of hypospadiacs (study group) and fathers of healthy children (control group)

Variables	Study group (n = 25)					Control group (n = 50)					Mann–Whitney test	
	$\bar{x}$	SD	Median	Minimum	Maximum	$\bar{x}$	SD	Median	Minimum	Maximum	Z	P
Sperm density (10^6 ml^{-1})	42.5	21.0	36.5	10	102	50.3	13.7	50.0	17	89	– 2.3	0.020
Sperm motility (%)												
Rapid, progressive	29.6	16.5	30.0	0	60	44.8	14.9	50.0	0	70	– 3.6	0.000
Slow or sluggish	18.4	7.5	20.0	10	40	13.6	6.9	10.0	10	40	– 3.2	0.001
Nonprogressive	20.0	7.1	20.0	10	40	15.8	6.7	20.0	0	30	– 2.9	0.020
Immotile	32.0	9.6	30.0	20	50	25.8	6.7	30.0	10	10	– 2.7	0.007

Table 3. Percentage occurrence of morphological defects in spermatozoa from fathers of hypospadiacs (study group) and fathers of healthy children (control group)

Sperm variables	Study group (n = 25)					Control group (n = 50)					Mann–Whitney test	
	$\bar{x}$	SD	Median (%)	Minimum	Maximum	$\bar{x}$	SD	Median (%)	Minimum	Maximum	Z	P
Large head	0.6	1.0	0	0	4	0.1	0.8	0	0	5	– 2.91	0.004
Small head	4.1	5.6	2	0	18	0.4	1.8	0	0	10	– 5.24	0.000
Amorphous head	9.8	4.3	10	2	19	11.8	6.7	10	0	38	– 1.05	0.290
Double head	0.2	0.6	0	0	2	0.1	0.9	0	0	2	– 2.01	0.044
Abnormal neck and midpiece	8.4	4.2	8	2	18	8.2	5.5	8	0	24	– 0.60	0.550
Multiple tail	0.2	0.8	0	0	4	0.2	1.4	0	0	10	– 1.76	0.080
Short, broken and coiled tails	2.7	2.4	2	0	8	3.1	4.2	2	0	25	– 0.24	0.810
Other defects	6.2	4.9	6	0	15	3.5	4.6	3	0	30	– 2.42	0.020

Discussion

The findings of the study reported here indicate a tendency towards abnormal sperm structure and function in the fathers of hypospadiacs in comparison with appropriate controls. Thus, our data confirms the study of Sweet *et al.* (1974) which was based on semen analysis of eight case and four control fathers. However, although the phenotypic manifestations of sperm dysmorphology and dysmotility have no direct relation to the genotype of spermatozoa and abnormalities of fetal development, the data presented here may explain our previous finding of a correlation between parental subfertility and a higher risk for hypospadias (Czeizel and Tóth, 1990) and support our hypothesis of relaxed selection.

There may be a connection between subfertility and hypospadias as a low production of androgen by the fetal testis is important in the aetiology of hypospadias, and the enzyme system required for fetal androgen production is determined by genetic and hormonal (mainly hCG) factors. Thus, the increased incidence of hypospadias may be related to the change of fertility pattern. In the past, subfertile fathers had no or few children. Now they may have the average number of children with a higher risk of hypospadias. These suppositions may explain the obvious inverse correlation between the fertility rate and the increased incidence at birth of hypospadias in both industrialized (for example, Sweden and Hungary) and developing countries (for example, Mexico and countries in South

America) (Källén *et al.*, 1986). The fall in the number of spermatozoa in adult men, the increasing incidence of male reproductive abnormalities and tumours may be explained mainly by the increased exposure of male fetuses *in utero* to xeno-oestrogen- or potent androgen-receptor antagonists (Sharpe and Skakkabaek, 1993; Kelce *et al.*, 1995) or to other environmental pollutions, for example, via the water supply (Ginsburg *et al.*, 1994). However, our hypothesis of relaxed selection offers a complementary explanation for the above trends.

The advantages of this study are the case–control approach and the blind evaluation of semen samples. The drawback of our study is the lack of recent high quality technology, for example, computer-assisted sperm analysis for the evaluation of motile cells. Thus, the results of Sweet *et al.* (1974) and those obtained in the present study need further confirmation.

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