Sperm morphological defects related to environment, lifestyle and medical history of 1001 male partners of pregnant women from four European cities

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BACKGROUND: Recently, differences in semen quality have been found among the partners of pregnant women from four European cities: Turku, Copenhagen, Edinburgh and Paris. METHODS: During this study, slides from the four centres were subjected to a centralized assessment of sperm morphology. The percentages of sperm defects were recorded using a multiple-entry classification enabling the calculation of the multiple anomalies index (MAI), which is the mean number of anomalies per abnormal sperm. The relationships between various sperm abnormalities and self-reported data on medical history, lifestyle and occupational factors were examined. RESULTS: Significant differences in the MAI and most of the sperm defects were found between the four cities, and more abnormalities were found in spring than in winter. An increase in some sperm abnormalities was related to medical treatment of the mother during pregnancy, higher birthweight and previous treatment for cryptorchidism. Significant variations of several sperm defects were related to stress, weekly working time, occupational posture and metal welding, suggesting directions for further exposure studies. CONCLUSION: The present study indicated that the detailed assessment of sperm abnormalities is a useful biomarker of the effect of various external factors which may qualitatively affect human spermatogenesis.

Key words: environmental factors/fertility/spermatogenesis/sperm morphology

Introduction

The morphology of seminal spermatozoa is the end result of a highly complex process of cellular modifications occurring during spermiogenesis. In humans, it leads to widely heterogeneous morphological patterns, with many cellular abnormalities which may be associated with sperm dysfunction. The morphological assessment of human spermatozoa—including the evaluation of the percentage of morphologically normal sperm and the determination of the incidence of various morphological abnormalities—has always been part of semen analysis in couples consulting for infertility [Rowe *et al.*, 1993; World Health Organization (WHO), 1999]. It is now well established that the percentage of normal sperm has prognostic value both *in vivo* (Jouannet *et al.*, 1988; Eggert-Kruse *et al.*, 1996; Bonde *et al.*, 1998) and *in vitro* (Kruger *et al.*, 1988; Toner *et al.*, 1995). It has also been reported that the proportion of some specific sperm abnormalities, and the mean number of abnormalities per abnormal spermatozoon [the Multiple Anomalies Index (MAI)] (Jouannet

et al., 1988; WHO, 1992, 1999) have a prognostic value both *in vivo* and *in vitro* (Jeulin *et al.*, 1986; Jouannet *et al.*, 1988).

Several studies suggesting secular and regional variations in human semen quality have recently been reported (Carlsen *et al.*, 1992; Auger *et al.*, 1995; VanWaeleghem *et al.*, 1996; Fédération CECOS *et al.*, 1997). The possible role of environmental and lifestyle factors in contributing to these variations has been widely discussed, and the studies have sparked off many debates and controversies because their results could be confounded by many factors (Jégou *et al.*, 1999). There has also been speculation that the observed variations could be due to exposure to environmental chemicals acting as endocrine disrupters (Jensen *et al.*, 1995; Toppari *et al.*, 1996). Most of the published data are on sperm concentration, being the semen characteristic most commonly assessed and the one least subject to methodological bias. In contrast, there are fewer studies reporting data on sperm motility and morphology. The assessment of these characteristics is more subjective by nature with an overall noticeable inter-technician and inter-laboratory variability (Neuwinger

et al., 1990; Cooper *et al.*, 1992; Matson, 1995; Ombelet *et al.*, 1998; Auger *et al.*, 2000). Moreover, sperm motility and morphology assessments are not fully standardized, despite WHO guidelines (WHO, 1992, 1999), which presents marked difficulties for multicentre studies.

To overcome the problems associated with retrospective studies on semen quality, a prospective multicentre study with a well-standardized protocol has been undertaken in four European cities: Turku (Finland), Copenhagen (Denmark), Edinburgh (UK) and Paris (France). The first results of this study provided clear evidence of geographical variations in sperm concentration and motility (Jørgensen *et al.*, 2001). During this study, microscope slide smears from semen samples collected in each of the four cities were centralized in Paris for Shorr staining and sperm morphology assessment. It has previously been reported that there were no significant geographical difference in normal sperm morphology (Jørgensen *et al.*, 2001). However, the various profiles of sperm abnormalities allowed an in-depth study of variation in the patterns of sperm abnormalities according to the geographical origin of the men as well as their medical history, environment and lifestyle, for which data were recorded by means of standardized questionnaires.

Materials and methods

Study population

The male partners of pregnant women were approached when they visited the antenatal care units and invited to participate in the study. The inclusion criteria were according to the standardized protocol described by Jørgensen *et al.* (Jørgensen *et al.*, 2001). Briefly, the eligibility criteria for each man were: 20–45 years of age at the time of invitation, living in the local referral area of the hospital to which he was recruited, and born in the country in which he was currently living. Furthermore, the current pregnancy had to be achieved by normal sexual intercourse, and not as a result of any treatment for subfertility or infertility. Participation in the study was accepted even if the man had a past history of urogenital disease or other diseases, as well as any treatment which may affect fertility. Altogether, 1082 men participated in the study; 275 from Turku, 349 from Copenhagen, 251 from Edinburgh and 207 from Paris. The inclusion period in each centre covered at least a full calendar year to take the possible influence of seasonal changes on semen parameters into account.

Semen samples

All the men were asked to abstain from ejaculation for at least 48 h before semen collection, but were not given any upper limit as we anticipated a reduction in the number of participants if such a limit was imposed upon this group of partners of pregnant women. For each man, a single semen sample was collected by masturbation and ejaculated into a clean collection tube. The assessment of sperm concentration and motility was made according to the then current WHO guidelines (WHO, 1992). Due to the wide inter-laboratory variations in sperm morphology assessment (Neuwinger *et al.*, 1990; Matson *et al.*, 1995), it was decided to perform this analysis centrally. Semen smears were prepared according to a standardized method in each centre, from a 10 µl drop of the sample, air-dried, then fixed for 1 h with a mixture of absolute ethanol (2/3) and acetic acid (1/3). Each centre sent the unstained coded smears to Paris. The smears were stained using an automatic stainer (Sakura DRS601,
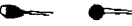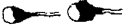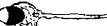
Bayer Diagnostics, Puteaux, France) which allows a homogeneity of staining between slides. The staining procedure was Shorr staining according to WHO manuals (WHO, 1992). For 81 (7.5%) of the 1082 men included in the study the smears could not be assessed, because they did not reach the centre, were broken during transportation or the identification code was not readable. Finally, 1001 slides (Turku = 261, Copenhagen = 294, Edinburgh = 239, Paris = 207) were randomly distributed to five technicians who assessed them blindly. The technicians involved in the study were chosen for their experience and accuracy in sperm morphology assessment as well as their good reproducibility and homogeneity in results, as revealed by regular internal quality controls following WHO recommendations (WHO, 1992). They had all worked in the laboratory for at least five years, and had on a daily basis assessed five to 10 smears made from semen samples of fertile and infertile men. The regular internal quality controls included the evaluation of intra- and inter-technician variability for the number of morphologically normal sperm and MAI. Just before the beginning of the study, the intra- and inter-individual coefficients of variations for these variables and the five technicians involved were <10%. No marked deviations of their quarterly means for both characteristics were observed.
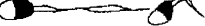
Method of classification of normal and abnormal spermatozoa

The percentages of morphologically normal spermatozoa and of spermatozoa showing various morphological anomalies were evaluated on 100 sperm at a final ×1000 magnification, according to the method described by David *et al.* and modified after the report of Jouannet *et al.* ((David *et al.*, 1975; Jouannet *et al.*, 1988). The modified classification of David *et al.* distinguishes morphologically normal sperm, seven abnormalities of the head, three abnormalities of the midpiece and five abnormalities of the tail (Figure 1). Normal sperm and all defects are defined by specific criteria. The originality of David's classification is that all abnormalities observed on each sperm cell are recorded thanks to a multiple-entry system. Thus, no abnormalities are underestimated in relation to another as shown on Figure 1. The methodology allows the calculation of the MAI (Figure 1). Other cellular elements (isolated tails, swollen sperm heads, white blood cells, immature germ cells, other cells and cellular debris) are also recorded, but they are not included in the count and they were not analysed in the present study.

Variables studied

All the men included in the study completed a standardized questionnaire containing information on previous or current general and urogenital diseases, lifestyle, occupation and on the current pregnancy (Jørgensen *et al.*, 2001). The possible influence of 35 recorded variables on detailed sperm morphology was studied. Two variables, city and season, were related to the general environment of the men, 10 variables concerned the medical history of the men (treatment of the mother during pregnancy, birth before term, birthweight, severe disease in the first year of life, treatment because one or both testicles were not in the scrotum, testicular trauma, history of Chlamydia infection, history of prostatitis, surgery for varicocele and surgery for inguinal hernia), seven variables were related to the lifestyle of the subject or his parents [smoking of parents during infancy, age of leaving school, alcohol (beer, wine and spirits) consumption, male and/or female partners smoking, diet exclusively based on organically produced food, satisfaction with sexual life, perception of stressful conditions] and 16 variables concerned the working conditions of the men (weekly working hours, working posture, metal welding, metal turning, metal degreasing, spraying and laying chemicals, house painting, cleaning with organic solvents, photo development, gluing,

Morphologically normal			34

	Tapered		0
Head Anomalies	Thin	◆	3
	Microcephalous		3
	Macrocephalous		2
	Multiple		0
	Abnormal post-acrosomal region	◆	26
	Abnormal or absent acrosome	◆	53
Midpiece Anomalies	Cytoplasmic droplet		2
	Thin		0
	Bent	◆	3
Tail Anomalies	Absent		2
	Short		0
	Irregular		0
	Coiled		9
	Multiple		0

Total number of isolated and associated anomalies = **T**	103
◆ **Multiple Anomalies Index (MAI)** = **T** / number of abnormal sperm (66 for 100 spermatozoa assessed)	1.56

Figure 1. Morphological classification of normal and abnormal human spermatozoa (David *et al.*, 1975, modified). The figure provides an illustration of each sperm defect recorded, these sperm defects as well as the morphologically normal spermatozoa being defined by specific criteria (David *et al.*, 1975). In this example, 100 spermatozoa have been assessed. Each abnormal spermatozoon with more than one morphological defect is recorded in all the corresponding defect categories, the methodology allowing the calculation of the Multiple Anomalies Index (MAI) (Jouannet *et al.*, 1988; WHO, 1992 and 1999). ◆ indicates the categories where an abnormal spermatozoon presenting four morphological defects (thin head, abnormal post-acrosomal region, abnormal or absent acrosome and bent midpiece) is recorded. Note that the total number of isolated and/or associated anomalies is greater than the number of abnormal sperm because all abnormalities of each spermatozoon are recorded in the multiple-entry grid.

plastic welding, working with anaesthetics or industrial lacquer or pesticides, working in laboratories, working at temperatures >50°C).

Statistical analysis
All statistics were run using the BMDP statistical software (Dixon, 1988; Statistical Solutions, Cork, Ireland). We first studied the possible confounding effects of the duration of sexual abstinence at collection and age of the men. Since there was no significant relationship between normal sperm morphology or the MAI and sexual abstinence or age, in each city and for all men, these factors were not used as adjustment variables. The equality of means values of the percentages of normal spermatozoa, the percentages of all morphological defects and of MAI between the groups was tested by a one-way analysis of variance (BMDP 7D software) every time there were more than two groups (the possible effect of city, season and alcohol intake) and taking in account cases of unequal variances (Brown-Forsythe test).

When rejecting the null hypothesis, Bonferroni's test, accounting for the variance estimate of all the groups, was used for pairwise mean comparisons.

The equality of means values of the percentages of normal sperm and the percentages of all morphological defects and of MAI when there were only two groups (dichotomic response for a qualitative variable, comparison of two qualitative variables or quantitative variables according to a given threshold) were tested by the distribution-free Mann–Whitney rank-sum test (BMDP 3D software).

Results
The mean and median MAI for all combined centres were both equal to 1.58 with 10th and 90th percentiles equal to 1.33 and 1.85 respectively, and the distribution of MAI was normal in each of the four cities. The mean MAI was significantly different among the cities (Table I), despite no significant difference in the percentage of normal spermatozoa (Jørgensen *et al.*, 2001), with the highest MAI value in Turku and the lowest in Paris. Except for the mean percentages of tapered heads and short tails, there were significant geographical variations in sperm defects with a different geographical pattern according to the category of anomaly assessed (Table I). The mean percentage of tail anomalies (the sum of the mean percentages of the five tail anomalies) was significantly higher in Edinburgh than in Paris ($P < 0.05$), Turku and Copenhagen (both $P < 0.01$) and there was a significant negative relationship between the percentage of grade a+b WHO motility (WHO, 1992) as reported in Jørgensen *et al.* (Jørgensen *et al.*, 2001) and the mean percentage of tail anomalies ($r = -0.19$, $P < 0.0001$ by Spearman's rank test, data not shown).

There were no seasonal variations in normal sperm morphology, while a number of abnormalities and MAI varied significantly according to season (Table II). Most of the significant differences in detailed sperm morphology were found between spring and winter.

The medical, lifestyle and occupational factors found significantly to modulate the mean percentages of at least two morphological characteristics are presented in Table III. An increase in some sperm abnormalities was related to medical treatment of the mother during pregnancy, higher birthweight and previous treatment for cryptorchidism. Significant variations of several sperm defects were related to stress, weekly working time, occupational posture and metal welding. Weak variations of the mean percentages of sperm defects were observed according to the following factors. Alcohol consumption: 0 versus 1–6 versus >6 units/week corresponded to a percentage of normal sperm of 48.3, 52.4 and 50.2% ($P = 0.017$), a mean percentage of cytoplasmic droplets (immature spermatozoa) of 1.3, 1.0 and 0.9 ($P = 0.04$) and a mean percentage of irregular tails of 0.6, 0.5 and 0.4 respectively ($P = 0.029$); age of leaving school: 1.3 versus 0.6% cytoplasmic droplets for ≥18 years old versus <18 years old ($P = 0.0009$); metal turning: daily ($n = 27$) versus never ($n = 829$) corresponded to a mean percentage of macrocephalous sperm of 0.9 and 0.3 respecively ($P = 0.027$); and finally, spraying and laying chemicals: daily ($n = 36$) versus never ($n = 814$) corresponded to a mean percentage of cytoplasmic droplets of 1.6 and 1.0 respectively ($P = 0.033$).

Table I. Sperm morphology in fertile men from four European cities: geographical variations

Characteristics	All $n = 1001$	Turku $n = 261$	Copenhagen $n = 294$	Edinburgh $n = 239$	Paris $n = 207$	P**
% Normal sperm	50.4*	52.0	49.4	49.9	50.3	NS
% Head anomalies						
Tapered	0.8*	0.8	0.8	0.8	0.9	NS
Thin	4.6	5.4[a]	5.2[b]	4.4[f]	3.0 [a,b,f]	< 0.0001
Microcephalous	2.4	2.8[a,f]	2.4	2.1[f]	2.0[a]	0.008
Macrocephalous	0.4	0.3	0.3	0.3	0.6	0.03
Multiple	0.5	0.6[a,f]	0.6	0.3[f]	0.2[a]	< 0.0001
Abnormal post-acrosomal region	21.1	19.6[f]	21.8	20.9	22.4[f]	0.004
Abnormal/absent acrosome	32.9	33.4[f]	34.9[a]	33.0	29.6[a,f]	0.0009
% Midpiece anomalies						
Cytoplasmic droplet	1.0	1.3[a,b,c]	0.4[a,d]	0.5[b,e]	2.0[c,d,e]	< 0.0001
Thin	0.10	0.13[f]	0.08	0.05[f]	0.07	0.01
Bent	6.0	6.0	6.7[a,b]	5.7[a]	5.5[b]	0.0009
% Tail anomalies						
Absent	1.6	1.4[f]	1.6	2.0[f]	1.7	0.03
Short	0.3	0.3	0.4	0.3	0.2	NS
Irregular	0.5	0.7[a,f]	0.5	0.5[f]	0.2[a]	< 0.0001
Coiled	6.6	5.5[a,f]	5.8[b]	8.4[a,b]	7.1[f]	< 0.0001
Multiple	0.4	0.5	0.4	0.3	0.3	0.003
Multiple anomalies Index	1.58	1.63[a,b,c]	1.60[c]	1.57[a,d]	1.51[b,c,d]14.1	< 0.0001

*Mean values of the percentages of normal sperm and abnormal sperm (for visual clarity, standard deviations are not presented); same superscripts in different columns denote significant differences of mean values by Bonferroni test at $P < 0.01$ ([abcde]) and $P < 0.05$([f]).
**Differences among cities were tested by ANOVA.

Table II. Sperm morphology in fertile European men: seasonal variations

Characteristics	All $n = 1001$	Season**				P***
		Autumn $n = 218$	Winter $n = 304$	Spring $n = 277$	Summer $n = 202$	
% Normal sperm	50.4*	51.2	50.0	50.2	50.2	NS
% Head anomalies						
Tapered	0.8*	0.8	1.0[f]	0.6[f]	0.9	0.049
Thin	4.6	4.9	4.1	4.9	4.7	NS
Microcephalous	2.4	2.1[a]	2.0[b]	2.9[a,b]	2.6[a]	0.0001
Macrocephalous	0.4	0.4	0.4	0.2	0.4	0.02
Multiple	0.5	0.3[a]	0.4[f]	0.6[a,f]	0.5	0.0007
Abnormal post-acrosomal region	21.1	20.6	21.3	20.9	21.8	NS
Abnormal/absent acrosome	32.9	32.3	32.6	34.2	32.3	NS
% Midpiece anomalies						
Cytoplasmic droplet	1.0	1.1[a]	1.3[b,c]	0.9[b]	0.5[a,c]	< 0.0001
Thin	0.10	0.06	0.08	0.10	0.08	NS
Bent	6.0	5.5	6.0	6.1	6.5	NS
% Tail anomalies						
Absent	1.6	1.5	1.5	2.0[f]	1.4[f]	0.03
Short	0.3	0.2	0.2[f]	0.4[f]	0.3	0.04
Irregular	0.5	0.4	0.3[a,f]	0.7[a]	0.6[f]	0.0003
Coiled	6.6	6.5	7.0	6.3	6.7	NS
Multiple	0.4	0.4	0.3	0.4	0.4	NS
Multiple anomalies index	1.58	1.56[f]	1.56[a]	1.62[a,f]	1.60	0.0019

*Mean values of the percentages of normal sperm and abnormal sperm (for visual clarity, standard deviations are not presented); **autumn: September–November, winter: December–February, spring: March–May, summer: June–August
Same superscripts in different columns denote significant differences of mean values by Bonferroni test at $P < 0.01$([a,b,c]) and $P < 0.05$([f]).
***Differences among seasons were studied by ANOVA.

The mean percentage of all sperm anomalies recorded in the fertile men studied was not significantly modulated by the following medical factors: birth before term, severe disease in the first year of life, testicular trauma, a history of Chlamydia infection or prostatitis, and surgery for varicocele or inguinal hernia. It was not modified by the following lifestyle factors: smoking by parents during infancy or by male and/or female partners smoking, diet exclusively based on organically produced food and satisfaction with sexual life. Finally, the mean percentage of all sperm anomalies was not found to be significantly modulated by the following occupational factors: house painting, cleaning with organic solvents, photo develop-

Table III. Sperm morphology in fertile European men: variations related with medical, lifestyle and occupational factors

Question	Unused data	Characteristics	Answer		P
Treatment during	Don't know $n = 730$		No $n = 85$	Yes $n = 186$	
		% Normal sperm	52.5[a]	48.3	0.042
		Abnormal post-acrosome region	19.0[a]	22.1	0.035
Weight of birth	Don't know $n = 135$		<3500 g[b] $n = 391$	≥3500 g[c] $n = 475$	
		Microcephalous	2.2	2.6	0.032
		Abnormal/absent acrosome	31.8	33.7	0.039
		Irregular tail	0.4	0.6	0.009
		Multiple tail	0.3	0.4	0.025
		MAI	1.57	1.61	0.005
Treatment because one or both testicles were not in the scrotum?	Don't know $n = 196$		No $n = 770$	Yes $n = 35$	
		% Normal sperm	50.5	40.5	0.0001
		Thin head	5.0	7.0	0.04
		Abnormal/absent acrosome	33.6	38.4	0.048
		Cytoplasmic droplet	0.7	1.7	0.0087
Level of stress in the last 3 months?	Don't know $n = 119$		Rarely $n = 736$	Every day $n = 146$	
		% Normal sperm	50.9	48.2	0.05
		Cytoplasmic droplet	0.9	1.3	0.047
		Irregular tail	0.5	0.3	0.036
		Coiled tail	6.5	8.1	0.0064
Hours/weeks for the last 3 months?	Don't know $n = 118$		>40[d] $n = 414$	≤40[e] $n = 469$	
		Thin head	4.1	4.9	0.0017
		Microcephalous	2.2	2.6	0.0015
		Multiple head	0.4	0.5	0.001
		Abnormal/absent acrosome	31.7	33.6	0.047
		Bent tail	5.6	6.3	0.0068
		Short tail	0.2	0.4	0.062
		Irregular tail	0.4	0.6	0.0006
		MAI	1.57	1.60	0.017
Working posture for the last 3 months?	Not used[f] $n = 875$		Standing $n = 92$	Seated[g] $n = 34$	
		% Normal sperm	52.3	47.5	0.10
		Abnormal post-acrosome region	19.9	27.4	0.0054
Metal welding in the last 3 months?	Missing $n = 148$		Never $n = 838$	Every day $n = 15$	
		Macrocephalous	0.4	0.7	0.026
		Coiled tail	6.7	9.1	0.10
		Multiple tail	0.4	0.9	0.0036

Differences studied by the Mann–Whitney test with a significant threshold level set at $P < 0.10$.
[a]Mean values of the percentages of normal sperm or abnormal sperm (for visual clarity, standard-deviations are not presented).
[b]Median = 3180 g; [c]median = 3830 g.
[d]Mean = 48; [e]mean = 33.
[f]No difference when seated at a table or front of a machine or variable posture.
[g]Exclusively seated in a vehicle.
MAI = multiple anomalies index.

ment, metal degreasing, gluing, plastic welding, working with anaesthetics, industrial lacquer, pesticide use and working in laboratories and at temperatures >50°C.

Discussion

The present study is the first one reporting the overall percentages of sperm defects in a wide population of fertile male partners of pregnant women (Table I), providing baseline data for future studies. It should be pointed out that several defects (tapered and macrocephalous heads, cytoplasmic droplets and short tails) were less frequent in the current group of male partners of pregnant women than in other groups of older fertile male candidates for semen donation or vasectomy, who would normally have a long period between the birth of the last child and semen collection (Schwartz *et al.*, 1984; Bujan *et al.*, 1988).

We found geographical differences for a majority of the sperm defects recorded as well as for the MAI. To our knowledge, this is the first report indicating subtle regional

changes in sperm morphogenesis. While such data obviously need to be confirmed by a further investigation in comparable groups of men, it is interesting to note that these differences were found in spite of an absence of differences in the overall proportion of normal sperm. Further investigations are warranted to confirm these findings, which suggest that even if the efficiency of spermiogenesis (in terms of the level of normal sperm release) is geographically stable in comparable healthy fertile men, qualitative differences in the morphogenesis of the head, midpiece and tail exist. We have no meaningful explanation for this phenomenon and the factors modulating these differences are probably numerous and complex.

It could be argued that these results and the other results of the study may be confounded by methodological factors. While we adopted the principle of a centralized assessment, pragmatically, the entire set of slides could not be read by a single technician. We believe this could not have influenced the results of the study since the smears were randomly distributed to the five technicians and blindly assessed, the procedure giving a similar weight to the reader effect in each comparison. Finally, due to the increased variance related to multiple readers, it can be postulated that, at the very worst, existing differences with the factors studied could not be shown for some defects while, on the contrary, the significant differences found, even with a probability level set at 0.10, reflected true differences.

We found significant relationships between some defects and season, but there was no seasonal variation in the percentage of morphologically normal sperm as previously reported in men from infertile couples (Ombelet *et al.*, 1996; Centola and Eberly, 1999). During the spring compared with the winter, there were less tapered and more microcephalous and multiple heads, shorter and more irregular tails, and fewer cytoplasmic droplets. Overall, we found more tail defects in the spring than in any other season, as recently reported for infertile patients in Rochester, UK (Centola and Eberly, 1999) while in contrast to the Rochester study, the percentage of cytoplasmic droplets was lowest during the summer. In addition, the mean number of sperm defects per abnormal spermatozoa was higher during the spring than during autumn and winter. While it is known that environmental temperature has an impact on human sperm production (Figa-Talamanca *et al.*, 1992; Thonneau *et al.*, 1998), we do not presently know if this can qualitatively affect spermiogenesis and further studies are warranted. It can be speculated that besides the possible impact of the environmental temperature, variations in light exposure and rhythmic changes of lifestyle may act as additional cofactors.

Semen analysis, including sperm morphology assessment, has been suggested to be a useful indicator of the factors in man's macro-environment which can modulate or damage spermatogenesis (MacLeod, 1974; Wyrobek *et al.*, 1983b). Experimental and occupational studies have shown unambiguously that the mammalian testis is highly vulnerable to numerous physical and chemical factors (Hacker *et al.*, 1981; Wyrobek *et al.*, 1983a; Steeno and Pangkahila, 1984) or more complex factors such as stress (Charpenet *et al.*, 1981; Fenster *et al.*, 1997; Yazawa *et al.*, 1999).

In the present study, we systematically investigated the possible role of a number of antecedent variables on detailed sperm morphology. The answers to the questions on the possible role of factors acting during testicular development and of the urogenital history of the men suggested that some events could have long lasting effects on sperm morphogenesis. The significantly lower percentage of normal spermatozoa and increased percentages of abnormal heads when the mother received treatment during pregnancy evoked the example of diethylstilboestrol (Gill *et al.*, 1979) which was widely used until the 1970s. Maybe other drugs administered during the crucial period of testis development could be implicated. Unfortunately, it was not possible to obtain more details on the type of treatments received.

Concerning the medical history of the man, the most obvious results were related to a history of treatment for one (or both) testi(cle)s not in the scrotum, whatever the anatomical position of the gonad, the nature of the treatment or the age at treatment. Our data suggested that treatments for cryptorchidism, or maybe cryptorchidism by itself, had a major impact on the efficiency of spermiogenesis as indicated by the markedly reduced proportion of normal spermatozoa. In contrast, acquired andrological diseases or iatrogenic factors seemed to have less impact. Notably, we found no effect of a history of Chlamydia infection. Intriguingly, the group of men with lower weights at birth had fewer sperm defects than the other men, when using the median birthweight as a threshold. Similar results were recently reported (Olsen *et al.*, 2000). There is presently no clear explanation of this phenomenon. Using a threshold set at 2500 g reinforced this result, which was not confounded by the duration of gestation (data not shown).

Some factors related to the sociological and lifestyle backgrounds of the men were found to possibly modulate sperm morphogenesis. Among them, perceived stress could have a noticeable impact on sperm morphogenesis for the men reporting daily exposure to mild stress. Similar results were previously reported for healthy volunteers (Giblin *et al.*, 1988) and subfertile men (Bigelow *et al.*, 1998). It should be pointed out that we found significantly more cytoplasmic droplets and coiled tails in men reporting being exposed to stressful conditions, suggesting that both defects could be putative markers of stressful conditions.

The men who had moderate alcohol habits (3.6 units per week on average) had less sperm defects than those drinking a lot (16.1 units per week on average), as previously suggested (Goverde *et al.*, 1995). Curiously, the men having moderate alcohol consumption had less defects than men who did not drink alcohol at all. We had no biologically meaningful explanation for this intriguing result, which warrants further studies since it is reminiscent of the 'French paradox' [the reported low rate of coronary heart diseases related to moderate alcohol consumption (Criqui and Ringel, 1994)]. There is a body of controversial literature on the effect of smoking on male fertility or semen quality (Ratcliffe *et al.* 1992; Vine, 1996). In the present study, we did not find any strong evidence for a relationship between smoking (smoking versus no smoking or comparisons between various levels of tobacco

intake) or passive exposure to smoke during early childhood and sperm defects.

The questions related to occupation referred to broad and heterogeneous occupational categories or to groups of agents (for example, solvents) rather than specific exposures. Although the study was carried out in an unselected population of partners of pregnant women—a marked difference to 'exposed–unexposed' studies including exposure measurements—and only a minority of men was exposed to possibly toxic occupational factors, we observed subtle differences in the number of sperm defects and some occupational 'exposures'. However, from the design of the study, methodological flaws may be suspected, but most of the significant differences were consistent with sparse previous literature, for example, on posture at work (Sas and Szöllözi, 1979; Figa-Talamanca *et al.*, 1996) or metal welding (Bonde, 1992; Bigelow *et al.*, 1998). Moreover, these general questions produced intriguing data which provided some trails for further studies; for example, the study indicated that more sperm defects were associated with the lowest rather than the highest weekly working times.

Finally, it should be pointed out that the present data on the relationships found between detailed sperm morphology and the male partners' environment, lifestyle and medical history should not be considered established before further confirmation. Due to the large number of statistical comparisons performed, the possibility cannot be excluded that a number of significant results may have arisen purely by chance. However, a number of these results were expected, based upon previous studies, for example the effect of birthweight, metal welding, treatment during pregnancy and the effect of stress.

In conclusion, the present data indicate that the detailed assessment of the incidence of sperm morphological abnormalities and MAI could be more useful than a simple evaluation of the percentage of normal spermatozoa to study the effect of external factors on human spermatogenesis. The study carried out in a large group of men with recently proven fertility suggests that the 'external milieu' may have subtle, complex and sometimes late impacts on the process of human spermiogenesis, and suggests a number of pathways for further exposure studies in humans and/or experimental studies.

Acknowledgements

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