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Regional differences in semen quality in Europe

Niels Jørgensen[1,7], Anne-Grethe Andersen[1], Florence Eustache[2], D.Stewart Irvine[3], Jyrki Suominen[4], Jørgen Holm Petersen[1,5], Anders Nyboe Andersen[6], Jacques Auger[2], Elizabeth H.H.Cawood[3], Antero Horte[4], Tina Kold Jensen[1], Pierre Jouannet[2], Niels Keiding[5], Matti Vierula[4], Jorma Toppari[4] and Niels E.Skakkebæk[1]

[1]Department of Growth and Reproduction, Rigshospitalet, The Juliane Marie Centre, Copenhagen, Denmark, [2]Université Paris V, Groupe Hospitalier Cochin, Paris, France, [3]MRC Reproductive Biology Unit, Centre for Reproductive Biology, Edinburgh, UK, [4]University of Turku, Institute of Biomedicine, Turku, Finland, [5]Department of Biostatistics, University of Copenhagen, Copenhagen, and [6]The Fertility Clinic, Rigshospitalet, Copenhagen, Denmark

[7]To whom correspondence should be addressed at: Department of Growth and Reproduction, Rigshospitalet, The Juliane Marie Centre, section GR-5064, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. E-mail: njcph@rh.dk

Recent reports have indicated a decrease in semen quality of men in some countries, and suggested regional differences. A study was undertaken of semen samples from 1082 fertile men from four European cities (Copenhagen, Denmark; Paris, France; Edinburgh, Scotland; and Turku, Finland). Semen analysis was standardized, inter-laboratory differences in assessment of sperm concentration were evaluated, and morphology assessment centralized. Lowest sperm concentrations and total counts were detected for Danish men, followed by French and Scottish men. Finnish men had the highest sperm counts. Men from Edinburgh had the highest proportion of motile spermatozoa, followed by men from Turku, Copenhagen and Paris. Only the differences between Paris/Edinburgh and Paris/Turku were statistically significant ($P < 0.003$ and $P < 0.002$ respectively). No significant differences in morphology were detected. A general seasonal variation in sperm concentration (summer 70% of winter) and total sperm count (summer 72% of winter) was detected. Semen quality of a 'standardized' man (30 years old, fertile, ejaculation abstinence of 96 h) were estimated. Typically, sperm concentrations ($\times 10^6$/ml) for winter/summer were: Turku 132/93; Edinburgh 119/84; Paris 103/73; and Copenhagen 98/69. These differences in semen quality may indicate different environmental exposures or lifestyle changes in the four populations. However, it remains to be seen whether such changes can account for these differences. These data may also serve as a reference point for future studies on time trends in semen quality in Europe.

Key words: fertile men/reference group/regional differences/semen quality

Introduction

Recent studies on secular trends in male reproductive health have provided conflicting evidence, with some investigations suggesting that sperm counts have declined significantly, whereas others have found no evidence of any change (Carlsen *et al*., 1992; Auger *et al*., 1995; Fisch *et al*., 1996; Irvine *et al*., 1996; Paulsen *et al*., 1996; Van Waeleghem *et al*., 1996; Vierula *et al*., 1996; Swan *et al*., 1997). However, a striking feature of much of the data is the appearance of regional differences in semen quality, which are at least as great as the possible secular trend. The possibility that these regional differences in sperm counts may be biologically meaningful has been suggested by the observation of higher fertility in Finland than in the UK, when assessed by time to pregnancy, a functional measure of fertility (Joffe, 1996). However, recently it was published that couple fertility had increased in the UK during the period 1961–1993, and this may have compensated for a possible decline in male fertility (Joffe, 2000).

The regional differences and adverse trends seen in male reproductive health clearly involve aspects other than spermatogenesis. It is generally agreed that the incidence of testicular germ cell cancer in adults is increasing and also shows great geographical variation (Adami *et al*., 1994; Forman and Møller, 1994). Furthermore, congenital malformations of the male genital tract, such as hypospadias and cryptorchidism may have increased in some geographical regions (World Health Organization, 1991; Ansell *et al*., 1992), although valid data are only available from very few areas.

The controversies of much of the published data are in part due to the fact that previous clinical studies on semen quality have dealt with selected groups of men: volunteers enrolled after advertisement (Irvine *et al*., 1996; Paulsen *et al*., 1996; Lemcke *et al*., 1997), candidates for vasectomy (Sheriff, 1983; Fisch *et al*., 1996), semen donor candidates (Leto and Frensilli, 1981; Auger *et al*., 1995; Bujan *et al*., 1996) or infertility patients (MacLeod and Wang, 1979). In many studies, historical data collected for other purposes have been used without close

attention to confounding factors which would be relevant to an analysis of secular or geographical trends. In most comparisons, the periods of abstinence from ejaculation, age and inter-laboratory differences have not been accounted for, to some extent because the underlying data were not obvious from the original reports. Although the present literature may suggest temporal and spatial differences in male reproductive health, it has also been pointed out that definitive evidence will only be provided by prospective studies (Irvine, 1996; Swan *et al*., 1997; Skakkebæk *et al*., 1998). Moreover, because prospective studies on secular trends in male reproductive health will be of a long-term nature, study of the apparent regional differences (if confirmed) could provide clues to the aetiology of the problem.

Previous data have indicated that Danish men may have low sperm counts (Bostofte *et al*., 1983) compared with Finnish men, who have high and unchanged sperm counts (Vierula *et al*., 1996). Furthermore, the sperm counts of French and Scottish men seem to have declined in recent years (Auger *et al*., 1995; Irvine *et al*., 1996b). Therefore, a cross-sectional study was undertaken focusing on the possible geographical differences in semen quality, by studying the male partners of pregnant women from Denmark (Copenhagen), France (Paris), Scotland (Edinburgh) and Finland (Turku) using co-ordinated standardized investigation procedures. Male partners of pregnant women were chosen as study subjects because they appeared to constitute well-defined, demographically comparable groups in each of the participating countries. Inclusion of infertile men would lead to less well-defined study groups which could not be compared reasonably. However, in countries having a military drafting system as in Denmark it is possible to investigate men from the general population (Andersen *et al*., 2000).

Materials and methods

Study population

The male partners of couples living in the local referral area of the four hospitals participating in the study were invited to participate. In Copenhagen, Paris and Turku all participants lived in urban areas. Pregnant women were approached during routine visits to the antenatal care units, and their husbands were invited to participate in the present study. In Edinburgh, subjects were recruited from the Lothian region, and thus men from both urban and surrounding rural areas were included. Subjects from Edinburgh were recruited from antenatal classes in hospitals or from outlying general practitioners' practices.

The eligibility criteria for each man were: 20 to 45 years of age at the time of invitation, residing in the local referral area of the hospital to which he was recruited, and being born in the country in which he was currently living. Further, the current pregnancy of the female partner 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 intracytoplasmic sperm injection, etc.). 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 illness, previous treatment for infertility or subfertility, unwanted pregnancy or prolonged waiting time to pregnancy were not exclusion criteria.

Couples fulfilling the eligibility were asked to participate in the study. Altogether, 1082 men participated in the study; 349 (participation rate 43%) from Copenhagen, 207 (participation rate 15%) from Paris, 275 (participation rate 19%) from Turku and 251 from Edinburgh. The inclusion process (the only one possible) in Edinburgh did not allow for calculation of a participation rate. Except in Paris, the participants received financial compensation for their travel expenses, and/or lost working hours, according to local custom and practice within this field. Information on age and previous diseases of the study population is summarized in Table I.

Inclusion period

The inclusion period in each centre covered at least a full calendar year in order to take the possible influence of seasonal changes on the semen parameters into account, and the men were examined as follows: Copenhagen, October 1996 to January 1998; Edinburgh, November 1996 to November 1997; Turku, November 1996 to June 1998; and Paris, January 1997 to January 1998, inclusive.

Questionnaires

On the day of attendance the men returned a completed standardized questionnaire which they had received in advance. The questionnaire included information on age, previous or current diseases and the number of previous pregnancies. Prior to the study, the standard questionnaire had been developed in English, and translated into Danish, Finnish and French. These translated questionnaires were back-translated to control for translation errors.

Semen samples

The semen samples were obtained by masturbation and ejaculated into a clean collection tube. WHO recommend that semen samples should be collected after a minimum of 48 h but not more than 7 days of ejaculation abstinence to standardize the influence this factor (World Health Organization, 1992). In this study, all men were asked to abstain from ejaculation for at least 48 h, but were not given any upper limit, as a reduction in the number of participants was anticipated if such a limit were to be imposed upon this group of partners of pregnant women. The period of ejaculation abstinence was calculated as the time between current and previous ejaculation as reported by the men.

In Paris and Edinburgh the semen samples were collected in the privacy of a room near the laboratories. Due to facility reasons, ~20% of samples from Turku and ~80% of samples from Copenhagen were collected at the men's home and delivered to the laboratory. If collected at home, the samples were protected from extremes of temperature ($<$20°C and $>$37°C) during transport to the laboratory. In the laboratories, the semen samples were kept at 37°C until analysed.

The analysis of semen samples was performed according to WHO conditions (World Health Organization, 1992), but further specified following assessment of inter-laboratory variation prior to the study (Jørgensen *et al*., 1997). Ejaculate volume was estimated by weighing the collection tube with the semen sample and subsequently subtracting the predetermined weight of the empty tube, and assuming that 1 ml of ejaculate weighs 1 g. Phase-contrast microscopy (positive phase-contrast optics) was used for the examination of fresh semen.

For the assessment of sperm motility, 10 µl of well-mixed semen was placed on a clean glass slide (which had been kept at 37°C), and covered with a 22×22 mm coverslip. The preparation was placed on the heating stage of a microscope (37°C), and immediately examined at a total magnification of ×400. The microscope field was scanned systematically, and the spermatozoa were classified as either motile (WHO motility classes A+B+C) or immotile (WHO motility class D), in order to report the proportion of motile spermatozoa. The

Table I. Age and frequency of self-reported, previous diseases of fertile men from four cities in Europe

Condition/disease	Copenhagen (n = 349)	Paris (n = 207)	Edinburgh (n = 251)	Turku (n = 275)	Between groups (P)
Mean (± SD) age (years)	31.5 ± 4.3	32.0 ± 4.4	32.5 ± 4.2	30.0 ± 4.5	<0.0005[a]
Median (5–95) age (years)	30.8 (25.4–39.6)	31.8 (25.4–40.2)	32.3 (26.0–39.4)	29.7 (22.9–39.1)	
	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	
First pregnancy[1]	46.4	37.0	70.9	48.0	<0.0005[b]
No conception within one year[2]	12.3	11.1	10.0	13.5	0.32[b]
Treated for infertility[3]	2.3	2.9	0.4	3.6	0.09[b]
Cryptorchidism[4]	4.3	5.3	2.8	1.8	0.16[b]
Testicular cancer[5]	0.3	0.0	0.0	0.0	0.55[b]
Varicocele[6]	2.9	2.4	1.2	1.5	0.44[b]
Testicular torsion[7]	1.1	1.0	0.8	0.0	0.39[b]
Inguinal hernia[8]	6.0	2.9	2.8	5.1	0.16[b]
Epididymitis[9]	2.6	2.9	2.6	1.8	0.84[b]
Chlamydia infection[9]	16.0	5.3	2.0	12.4	<0.0005[b]
Gonorrhoea[9]	4.3	5.8	2.0	2.5	0.11[b]
Orchitis[9]	0.3	0.0	0.0	0.0	0.58[b]
Diabetes[9]	0.3	0.5	1.2	0.0	0.22[b]
Thyroid disease[9]	0.0	1.0	0.4	1.1	0.25[b]

[1]Men who had not previously caused a pregnancy, neither with present nor with previous partners.
[2]Men who had experienced at least 1 year of unprotected intercourse with a female partner without her becoming pregnant.
[3]Men who had been treated for fertility problems in the past.
[4]Men who had previously been treated for cryptorchidism, either by hormonal treatment, surgery or combination.
[5]One man from Denmark had been treated for testicular cancer.
[6]Men who had previously been diagnosed as having varicocele, irrespective of whether treated or not.
[7]Men who had previously been treated for torsion of either one or both testicles.
[8]Men who had previously been diagnosed as having an inguinal hernia, irrespective of whether treated or not.
[9]Men who had previously been diagnosed as having the disease. No information regarding treatment.
[a]Kruskal–Wallis test.
[b]Chi-square test.
(5–95) = 5th–95th percentile.

motility assessment was repeated on a second 10 µl aliquot of semen, and the average value was calculated for both samples.

For the assessment of sperm concentration, each semen sample was thoroughly mixed for at least 10 min in a rotation device. An aliquot of the sample was put into the diluent using a positive displacement pipette and mixed for a further 10 min. The diluent consisted of 50 g $NaHCO_3$, 10 ml 40% formaldehyde and distilled water up to 1 litre. The sperm concentration was assessed using haemocytometers (Bürker-Türk chamber in Copenhagen and Turku; Neubauer chamber in Edinburgh; Thoma chamber in Paris). One drop of the diluted specimen was transferred to each chamber of the haemocytometers, which were allowed to stand for 5 min in a humid chamber before the cells were counted at a total microscope magnification of ×400. Only spermatozoa with tails were counted.

Smears for morphology evaluation were made. The thickness of the smears was varied according to the sperm concentration in each sample. The smears were air-dried, fixed for 1 h with a mixture of absolute alcohol (2/3, v/v) and acetic acid (1/3, v/v), and then sent to Paris for a modified Shorr stain (World Health Organization, 1992), and assessment of sperm morphology according to criteria described previously (David *et al*., 1975).

External quality control of sperm concentration assessment

All centres participated in an external quality control (QC) programme for sperm concentration assessment in the period January 1997 to June 1998. Briefly, each month five blinded samples were sent from the Copenhagen centre to the other laboratories. Fresh samples from normal semen donors were preserved by addition of 10 µl of a 3 mol/l (~20%) sodium azide solution per 1 ml of the ejaculate (after liquefaction). This procedure was used in order to obtain undiluted samples, since the dilution step is considered to be an important source of variation when sperm counting is performed. Each centre received 600 µl of semen. To obtain a sufficient volume, ejaculates from two or more donors were mixed, and the samples were sent by mail in 1 ml cryotubes. Thus, all centres performed sperm counting according to their techniques described above, 2–7 days after the semen preparation was performed, including Copenhagen. The results were reported to the Copenhagen centre for statistical analysis.

Physical examination

Physical examination of each participant was performed on the day of the delivery of his semen sample. Evaluations of testes disposition, varicocele and Tanner stages of pubic hair were performed with the men in standing position. For assessment of testis size all examiners used the same type of wooden orchidometer (Pharmacia & Upjohn, Denmark).

Data acquisition

Standardized questionnaires, record forms for physical examination and semen analyses were labelled with identification (ID) numbers. The information linking ID-numbers to personal data were kept separately in each centre so as to preserve confidentiality. Information from the questionnaires, and results of semen analysis and physical examination were sent to Copenhagen and entered into a centralized database.

Statistical analysis

Sperm concentrations and total sperm counts were best normalized by cubic root transformation before analysis to correct for the markedly skewed distribution. Multivariate regression analyses were carried out to compare differences between centres. In these analyses the general level of each centre was estimated while adjusting for known confounders, including age, abstinence time and season. Age and abstinence time entered the model as piecewise linear functions

Table II. Semen parameters of fertile men from four cities in Europe

Parameter	Copenhagen (n = 349)[a]		Paris (n = 207)[a]		Edinburgh (n = 251)[a]		Turku (n = 275)[a]	
	Mean ± SD	Median (5–95)	Mean ± SD	Median (5–95)	Mean ± SD	Median (5–95)	Mean ± SD	Median (5–95)
Semen volume (ml)	3.8 ± 1.7	3.6 (1.4–6.7)	4.2 ± 2.0	3.9 (1.6–8.2)	3.9 ± 1.8	3.6 (1.4–7.6)	4.1 ± 1.6	3.9 (2.1–7.4)
Sperm conc. ($\times 10^6$/ml)	77 ± 66	61 (10–207)	94 ± 72	74 (15–231)	92 ± 63	77 (15–222)	105 ± 73	82 (19–262)
Total spermatozoa ($\times 10^6$)	276 ± 240	215 (32–795)	385 ± 350	293 (46–1177)	343 ± 279	280 (58–925)	412 ± 312	328 (71–1063)
Motile spermatozoa (%)	60 ± 12	61 (40–79)	56 ± 12	55 (40–78)	67 ± 10	68 (51–83)	66 ± 10	66 (49–81)
Normal morphology (%)	49 ± 15	51 (23–71)	50 ± 16	54 (20–72)	50 ± 15	52 (21–71)	52 ± 15	53 (24–74)

Results are based on raw data reported from each city. Thus, possible confounders are not taken into account.
[a]For one of the 349 men from Copenhagen, motility was not evaluated. Due to broken slides, morphology was assessed on 294, 207, 239 and 261 semen samples of men from Copenhagen, Paris, Edinburgh and Turku respectively.
Some men had a period of ejaculation abstinence less than the recommended 48 h (Copenhagen 19%, Paris 3%, Edinburgh 13% and Turku 7%), but data are based on semen samples from all men.
(5–95) = 5th–95th percentile.

(linear splines); for example, one straight line for abstinence below 48 h, another straight line for abstinence periods 48–96 h, etc. Season entered the model as a categorical variable allowing each of four seasons to have a different level (spring, March–May; summer, June–August; autumn, September–November; winter, December–February). The final models were subjected to standard checks of the residuals. Natural logarithmic transformation gave models in which centre differences and effects of covariates are more easily interpretable. This alternative model approximates very closely the model obtained transforming with the cubic root and is used when reporting centre differences (see Table IV). The percentages of motile spermatozoa were logit-transformed and analysed in a multivariate regression model while adjusting for age, abstinence time, seasonal variation, and the delay from time of ejaculation to assessment of motility. The percentages of morphologically normal forms were arcsine-square root-transformed and also analysed in a multivariate regression model adjusted for age, abstinence time and seasonal variation.

Between-centre differences in men's age, previous medical history, previous pregnancies and other questionnaire information were tested with the Pearson chi-square test. The non-parametric Kruskal–Wallis test was used for testing differences between centres regarding the interval between ejaculation and start of analysis. The within-centre differences for semen qualities (estimated on transformed/untransformed data as described above) between subgroups (e.g. previous *Chlamydia* infection versus never *Chlamydia*) were also tested by one-way analysis of variance (ANOVA). The statistical analyses were performed using the statistical packages SAS version 6 and SPSS version 8.0. The *P*-values presented have not been subjected to any corrections to account for multiple testing.

Results

Semen parameters of fertile men from four cities in Europe are summarized in Table II. These results are based upon the raw data obtained in each city, and therefore possible confounders are not taken into account in this table. Thus, no statistical comparisons are given at this point. Overall, the period of abstinence differed between men from the four centres ($P < 0.005$). The Danish men reported the shortest abstinence period (mean/median; 81/64 h) followed by Finnish men (109/70 h), Scottish men (156/82 h) and French men, who had the longest period of abstinence (157/96 h). The age of the participating men differed significantly between men from the four cities ($P < 0.0005$). Ages were as follows (mean/median): Copenhagen 31.5/30.8 years; Paris 32.0/31.8 years; Edinburgh 32.5/32.3 years; and Turku 30.00/29.7 years (see Table I).

Table III. Inter-laboratory differences in assessment of sperm concentration observed from quality control study[a]

Location	Difference[b] (%) (CI)	*P*
Turku/Copenhagen	109 (99–120)	0.06
Turku/Edinburgh	98 (72–133)	0.3
Turku/Paris	106 (95–118)	0.9
Copenhagen/Edinburgh	90 (79–102)	0.09
Copenhagen/Paris	97 (91–103)	0.4
Edinburgh/Paris	108 (91–130)	0.4

[a]Note that all the pair-wise differences between the centres were statistically non-significant.
[b]Difference = relative difference in assessment of sperm concentration of quality control samples. For example, a difference Turku/Copenhagen of 109% shows that the centre in Turku assessed the concentration 9% higher than the Copenhagen centre. However, the confidence interval and the *P*-value indicate that the difference is non-significant.
CI = 95% confidence interval.

Seasonal variations in sperm concentrations and total sperm counts were detected, with the highest counts being observed during the winter season, and the lowest during summer season in all four centres, with spring and autumn values being in between. No seasonal variation was detected for sperm motility or sperm morphology.

The delay from time of ejaculation to assessment of motility was as follows (mean/median): Copenhagen 83/80 min; Paris 37/35 min; Edinburgh 36/35 min; and Turku 42/40 min ($P < 0.0005$).

The results of the quality control study of sperm concentration showed no significant differences between the four centres ($P = 0.17$). Additionally, no significant differences were detected when comparing the centres pair-wise (Table III). Therefore, no correction for laboratory differences was included in the statistical analysis.

The relative differences in semen qualities between the centres are shown in Table IV. These results were obtained following logarithmic transformation of the raw data and control for period of abstinence, men's ages, season and also

Table IV. Relative differences [% (CI)][a] in semen parameters of fertile men from four cities in Europe[b]

Location	Sperm conc.	*P*	Total sperm count	*P*	Motile spermatozoa	*P*	Normal morphology	*P*
Turku/Copenhagen	135 (117–155)	0.00002	148 (127–172)	0.0001	121 (100–147)	0.08	103 (100–105)	0.07
Turku/Edinburgh	110 (99–123)	0.07	120 (105–136)	0.005	98 (82–117)	0.82	103 (100–105)	0.09
Turku/Paris	128 (111–147)	0.0008	142 (121–166)	0.0001	147 (122–177)	0.003	102 (99–105)	0.20
Copenhagen/Edinburgh	82 (69–98)	0.03	81 (66–99)	0.03	81 (66–98)	0.06	100 (97–103)	0.98
Copenhagen/Paris	95 (75–119)	0.64	96 (70–131)	0.80	121 (99–148)	0.09	99 (96–103)	0.72
Edinburgh/Paris	116 (97–138)	0.11	118 (97–144)	0.09	150 (125–180)	0.002	100 (96–103)	0.75

[a]Difference = relative differences in semen qualities. For example, difference Turku/Copenhagen of 135% for sperm concentrations shows that the fertile men from Turku have a 35% higher sperm concentration than the fertile men from Copenhagen, and the difference Copenhagen/Edinburgh of 82% for sperm concentration shows that the fertile men in Copenhagen have a concentration of 82% compared with men from Edinburgh, i.e. 18% below the men from Edinburgh. For sperm motility, the ratio of motile to immotile spermatozoa is 21% higher in Turku than in Copenhagen. The percentage of morphologically normal spermatozoa is 3% higher (52 versus 49%) higher in Turku than in Copenhagen.
[b]Results are based on transformed data, taking confounders into account.
Results are corrected for the confounders of age, abstinence period, season and for motility additionally for delay from time of ejaculation to assessment.
See text for further explanation.
CI = 95% confidence interval.

Table V. Calculated expected semen parameters of a 30-year-old, recently proven fertile man, having an ejaculation abstinence period of 96 h and motility assessment performed 30 min after ejaculation. Estimates are based on regression analysis of cubic root-transformed data

Parameter		Copenhagen		Paris		Edinburgh		Turku	
		Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Sperm conc.	($\times 10^6$/ml)	98	69	103	73	119	84	132	93
	CI	(83–115)	(58–82)	(88–121)	(62–86)	(101–141)	(71–100)	(112–155)	(78–111)
Total spermatozoa	($\times 10^6$)	374	269	389	280	461	332	552	397
	CI	(314–445)	(226–319)	(329–461)	(235–334)	(388–549)	(278–396)	(467–655)	(330–478)
Motile spermatozoa	(%)	63	63	58	58	68	68	67	67
	CI	(59–67)	(59–67)	(55–62)	(55–62)	(65–71)	(65–71)	(64–70)	(64–70)
	Ejaculate ($\times 10^6$)[a]	236	169	226	162	313	226	370	266
Normal morphology	(%)	47.7	47.7	50.3	50.3	49.8	49.8	52.3	52.3
	CI	(47.3–51.7)	(47.3–51.7)	(47.9–52.6)	(47.9–52.6)	(47.6–51.9)	(47.6–51.9)	(50.3–54.2)	(50.3–54.2)
	Ejaculate ($\times 10^6$)[a]	178	128	196	141	230	165	289	208

[a]Total number of motile or morphologically normal spermatozoa per ejaculate. Neither any general seasonal variations nor general effect of men's ages could be detected for relative number of motile and morphologically normal spermatozoa (of the groups of men in our study), and thus these figures are the same for all seasons, whereas the total number differed between the seasons due to different total sperm counts.
See text for further explanation.
CI = 95% confidence interval.

for sperm motility for delay from time of ejaculation to assessment of motility.

Table V shows, for each of the four cities, the expected calculated semen quality of a recently proven fertile, 30-year-old man, having an ejaculation abstinence period of 96 h. Examples for both the winter and summer seasons are given; these values are constructed based on the results from the regression analyses after taking the confounders into account.

Tables IV and V indicate that the lowest sperm concentrations and total sperm counts were detected among the Danish men, followed by French and Scottish men, while Finnish men had the highest numbers. For the proportion of motile spermatozoa the ranking was slightly different, with men from Edinburgh having the highest value, followed by men from Turku, Copenhagen and Paris. With regard to motility, only the differences between Paris versus Edinburgh and Paris versus Turku were statistically significant at a 5% level. The highest percentage of morphologically normal spermatozoa appeared in men from Turku, followed by men from Paris and Edinburgh; men from Copenhagen had the lowest proportion of morphologically normal forms. However, none of these differences in morphology was statistically significant.

The parity (i.e. whether the men were involved in a pregnancy for the first time versus subsequent pregnancies) differed between the four investigated groups of men ($P < 0.0005$), as those involved in a pregnancy for the first time were as follows: Copenhagen 46%; Paris 37%; Edinburgh 71%; and Turku 48% (see Table I). However, no difference in sperm counts between those involved in first versus subsequent pregnancies were found (e.g. $P = 0.65$, for sperm concentration).

Self-reported, previous subfertility (waiting time to pregnancy of more than 1 year), treatment for infertility, cryptorchidism, testicular cancer, varicocele, testicular torsion, inguinal hernia, epididymitis, orchitis, gonorrhoea, diabetes and thyroid diseases did not differ between the groups of men from the four cities (see Table I). However, a high percentage of Finnish (12.4%) and Danish (16.0%) men reported a previous chlamydial infection compared with French (5.3%) and Scottish (2.0%) men ($P < 0.0005$). Within-centre com-

parisons did not reveal any differences between those reporting previous *Chlamydia* infection and those who had not had *Chlamydia* regarding period of ejaculation abstinence, semen volume, sperm concentration, total sperm count, proportion of motile spermatozoa and morphologically normal spermatozoa. Detailed information on how the diagnosis was established or on treatment was not available.

Physical examinations showed that none of the participants suffered from severe genital abnormalities, e.g. micro-penis or micro-testis, and all men (except for one from Copenhagen and three from Edinburgh) had both testes in the scrotum. The testicular volumes (mean of left and right testicles) were (median): Denmark 23.5 ml; Edinburgh 23.0 ml; France 22.5 ml; and Finland 23.0 ml. In all four groups of men, the right testis appeared to be larger than the left testis.

All Danish and Finnish men, 98.4% of Scottish men and 89.1% of French men were found to have an adult pubic hair distribution (i.e. Tanner stage 5). Differences in the number of men with varicoceles were detected at the physical examination for the four groups of men: Copenhagen 1%; Paris 4%; Edinburgh 5%; and Turku 3%.

Discussion

In this coordinated cross-sectional study, significant differences were detected in semen quality between well-defined groups of men from four European cities. The Finnish (Turku) men had the highest sperm counts, followed by men from Scotland (Edinburgh) and France (Paris), while Danish (Copenhagen) men had the lowest sperm counts. None of the differences detected in morphology reached statistical significance, but did indicate that Danish men appeared to have the lowest proportion of normal spermatozoa while Finnish men the highest proportion. For the motility, the pattern changed somewhat. Nevertheless, Danish and French men had the lowest proportion of motile spermatozoa. All four groups of men were investigated according to the same protocol; inter-laboratory differences in assessment of sperm concentrations were controlled by an external quality control programme to assure that the detected differences were not due to inter-laboratory variation (including the use of different types of haemocytometer) in assessment of sperm concentration. Additionally, comparisons between the centres were controlled for known confounders. Parity could not explain the detected differences, as no difference in sperm quality between men involved in first versus subsequent pregnancies could be detected. Previous diseases, including fertility problems, did not differ between the four groups of men; the vast majority of the men had normal testis sizes, both testicles in the scrotum and a normal penis, without hypospadias. Participation rates varied between centres and were not particularly high, which is always the case when men are requested to deliver semen samples. However, the volunteers would appear to be normal men belonging to comparable groups, and therefore we believe that our findings reflect genuine differences in sperm counts between men from these four cities. Moreover, in the interpretation of our findings it must be kept in mind that the study subjects were partners of pregnant women, inevitably implying that subfertile men were under-represented, while infertile men were not included at all. However, the presented data may serve as a reference point for future studies on time trends in semen quality in Europe.

Based upon the results, it was possible to calculate expected values for a standardized 30-year-old fertile man from each of the four cities. In Table V, the confidence intervals of the estimates are given in order to state the 'certainty' of the given levels. These confidence intervals should not be interpreted as 95% confidence intervals for the populations.

The magnitude of the seasonal variations in sperm counts emphasized the importance of controlling for this factor. An apparent difference in sperm counts of ~30% occurred from winter to summer in all four centres. Some previous studies have also detected seasonal variations, and consistently the lowest sperm counts were detected during the summer season and highest during either autumn or winter seasons (MacLeod and Heim, 1945; Tjoa *et al*., 1982; Spira, 1984; Maier *et al*., 1985; Gyllenborg *et al*., 1999). These studies were performed either in Europe or the USA, and some included men of known fertility while others included men of known subfertility. However, other studies have been unable to detect seasonal variation. For example, an Australian study of 'normal' men (Mallidis *et al*., 1991) and a Belgian–South African study of infertile men (Ombelet *et al*., 1966) did not detect any seasonal variation. The present study was cross-sectional in nature, as were the majority of the previous published studies regarding seasonality. The possible seasonal variation should be investigated by longitudinal studies. Until convincing results of such studies are published, it is recommended that seasonality be included equally as other confounders such as abstinence period and age.

The differences in the proportions of motile and morphologically normal spermatozoa between men from the four cities gave a different ranking than the sperm counts, but the majority of differences in these parameters were not statistically significant. In spite of attempts to standardize motility assessments between laboratories, the inter-technician variation may still be considerable and may account for some of the differences. For the morphological assessment, staining and evaluation was centralized, and thus the results are not likely to be explained by technical reasons. Nevertheless, calculating the total number of morphologically normal forms per ejaculate revealed the same ranking as the sperm counts: Danish men had the lowest number, followed by men from Edinburgh and France, while Finnish men had the highest absolute number of normal forms.

It is interesting to consider our finding of geographical differences and previous reports on time trends in semen quality in connection with similar patterns in testicular cancer. The incidence of this disease is rising in almost all countries, and is five times higher among Danish men than among Finnish men (Adami *et al*., 1994; Forman and Møller, 1994), who correspondingly, in the present study, had a much better sperm count. The adverse relationship between sperm count and the risk of testicular cancer is not only apparent in cohort studies, but is also seen in individuals (Møller and Skakkebæk, 1999). The synchronized trends in semen quality and testicular

cancer may reflect common prenatal risk factors (Skakkebæk *et al*., 1987, 1998; Møller and Skakkebæk, 1999). It is generally accepted that the precursor cells of testis cancer—the carcinoma in-situ germ cells—have several characteristics of fetal germ cells, including stem cell markers (Skakkebæk *et al*., 1987; Damjanov, 1991; Jørgensen *et al*., 1995; Rajpert-De Meyts *et al*., 1998). They are thought to arise perinatally as a result of a carcinogenic change of the primordial germ cells. Also, epidemiological studies support the hypothesis of a fetal origin: both testicular cancer and semen quality have been linked to birth cohort effects. Thus, men born in Scandinavia during the Second World War had a relatively lower risk of developing testicular cancer in adult life than men born before or after the war (Møller, 1993; Adami *et al*., 1994). In addition, two studies have indicated that sperm counts seem to decline with a more recent year of birth (Skakkebæk *et al*., 1987; Irvine *et al*., 1996). A possible theory is that exogenous factors which interfere with the function and multiplication of the fetal Sertoli cell may be to blame for a syndrome of reduced sperm count, hypospadias, undescended testis and testicular cancer (Sharpe and Skakkebæk, 1993; Bergman *et al*., 1996). In this respect, it is noteworthy that the gradient in the incidence of hypospadias between Denmark and Finland seems to parallel that of testicular cancer (Toppari *et al*., 1996).

In conclusion, this international coordinated study of fertile men has revealed significant differences in semen quality between men from four European cities. Although genetic differences cannot entirely be ruled out as causes, a more likely explanation for these findings seems to be differences in lifestyle or other environmental factors. Recently, endocrine disrupters have been in focus as possible aetiological, environmental factors. Whatever the causes are, the differences in male reproductive health between countries in Europe are considerable, and the fact that several aspects of reproductive function are involved (sperm production, testicular cancer, hypospadias) suggests that the differences are robust. Considering the great importance of reproductive health, these results should be followed by a close look at possible environmental and lifestyle differences between countries with the greatest differences in male reproductive health.

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