Human Reproduction vol.12 no.4 pp.740–745, 19997

Evidence for regional differences of semen quality among fertile French men

Fédération Française des CECOS[1], Jacques Auger[2,3] and Pierre Jouannet[2]

[1]CECOS (Centres d' Etude et de Conservation des Oeufs et du Sperme humains), [2]CECOS Paris-Cochin, Service d' Histologie et Biologie de la Reproduction, Hôpital Cochin, Université Paris V, Paris, France

[3]To whom correspondence should be addressed at: CECOS Paris-Cochin, Groupe Hospitalier Cochin, 75014 Paris, France

The world literature on human semen quality indicates apparent geographical differences but these might primarily depend on variations among studies for subject recruitment strategy, semen analysis or data processing methods. A retrospective analysis on the quality of semen from 4710 healthy unselected fertile men, who were candidate semen donors to sperm banks in university hospitals in eight different French areas during the period 1973–1993, was undertaken. In these centres, all the men were referred under the same guidelines and all semen samples were analysed using similar methodologies. Significant differences were found between centres for seminal volume, sperm concentration, total number of spermatozoa in the ejaculate and percentage of motile spermatozoa (all $P < 0.0001$). Multiple regression analysis accounting for the age, sexual abstinence before semen collection and year of semen collection also showed regional differences: compared to Paris, the seminal volume was higher in Caen ($P < 0.001$) and lower in Toulouse ($P < 0.01$), the total number of spermatozoa was higher in Lille ($P < 0.001$) and lower in Toulouse ($P < 0.05$) and the percentage of motile spermatozoa was higher in Bordeaux and lower in Tours (both $P < 0.001$). This is the first study providing evidence for regional differences in the human semen quality.

Key words: geographical differences/human/semen quality

Introduction

The meta-analysis of Carlsen *et al.* (1992), suggesting a possible decline in human semen quality, has stimulated extensive discussion and controversy (Bromwich *et al.*, 1994; Olsen *et al.*, 1995). Recently, it was reported that the mean sperm concentration of Finnish men may be higher (140×10^6/ml) than elsewhere in Europe (Vierula *et al.*, 1996). Furthermore, mean sperm concentrations of men from four different regions of the USA were found to vary significantly (Fisch *et al.*, 1996; Paulsen *et al.*, 1996). Such differences might be related to ethnic, genetic or environmental factors (Danish Environmental Protection Agency, 1995; Adamopoulos *et al.*, 1996). However, these apparent geographical variations could also be affected by differences in subject recruitment strategy, method of semen analysis or data processing.

The same guidelines for recruiting fertile male volunteers for semen donation are applied in the French CECOS sperm bank network established in university hospitals, and similar methodologies are used for semen analysis. This presents a unique situation for the comparison of the semen quality of men living in different geographical areas. The semen characteristics of 4710 unselected fertile men referred to eight CECOS centres, during the period 1973–1993, were analysed retrospectively.

Materials and methods

Subjects

All the volunteers for semen donation in the eight CECOS centres before 1994 were included, except for those known to have infertile brothers, since it has been reported that the characteristics of their semen differed from those of other fertile men (Auger *et al.*, 1995). The men were healthy, had fathered at least one child and 98% were Caucasian. The numbers of men included from each centre and the period of study are shown in Table I. The relative proportions of different socio-professional categories were similar in different centres. When the men collected several semen samples, their semen characteristics were better than those of the men who attended only once. It was therefore decided to include only the first ejaculate from each volunteer to avoid a selection bias. All ejaculates were collected by masturbation at the laboratories after a recommended duration of sexual abstinence of 2–5 days.

Semen analysis

The methodologies for semen analysis were similar in the eight laboratories and there was no technical change with time in any laboratory. In seven the seminal volume was measured directly on a graduated pipette, whereas in Paris seminal volume was determined by weighing assuming that 1 ml = 1 g. Sperm concentration was determined using a haemocytometer in seven laboratories; in Rennes, however, a Makler chamber was used (Makler, 1980). Semen samples were diluted 1:20 with distilled water containing formalin and well mixed before transferring a drop to the two chambers of the haemocytometer. After about 5 min allowing for the sedimentation

CECOS centres participating in the study: CECOS Paris Cochin-Ile de France (J.Auger, P.Jouannet), Caen-Basse Normandie (A.Sauvalle), Grenoble-Alpes (M.Servoz-Gavin), Toulouse-Midi Pyrénées (L.Bujan, A.Mansat), Bordeaux-Aquitaine (J.J.Berjon, J.Morand), Lille-Nord (I.Helin, P.Saint Pol), Rennes-Ouest (D.Le Lannou) and Tours-Centre (C.Barthelemy, D.Royère). The study was supported by EU contract BMH4-CT96-0314 and a research grant (1752) from Direction des Recherches, Etudes, et Technologies, Ministère de l'Education Nationale.

Table I. Age of fertile men and duration of sexual abstinence prior to semen collection in the eight regions of France analysed: mean values ± SD (25th–75th percentile) and number of men for which the value was recorded (%)

Region (period of study)	Total no. of men	Age (years)		Duration of sexual abstinence (days)	
		Mean ± SD	*n* (%)	Mean ± SD	*n* (%)
All regions	4710	35 ± 6 (30–38)	4654 (99)	4.0 ± 5.2 (3.0–4.0)	3347 (71)
Paris (1973–1993)	1396	34 ± 6 (29–37)	1396 (100)	4.6 ± 4.4 (3.0–5.0)	1094 (78)
Caen (1978–1993)	226	36 ± 6 (31–40)	226 (100)	4.2 ± 2.0 (3.0–5.0)	95 (42)
Grenoble (1978–1993)	277	37 ± 8 (32–42)	277 (100)	3.7 ± 4.2 (2.5–4.0)	277 (100)
Toulouse (1977–1993)	371	35 ± 6 (31–39)	368 (99)	5.0 ± 5.9 (3.0–5.0)	128 (35)
Bordeaux (1984–1993)	330	34 ± 6 (30–38)	330 (100)	3.0 ± 1.1 (3.0–3.0)	330 (100)
Lille (1982–1993)	402	35 ± 6 (30–39)	378 (94)	2.4 ± 1.1 (2.0–3.0)	388 (97)
Rennes (1977–1993)	1007	35 ± 6 (31–38)	988 (98)	3.6 ± 2.6 (2.0–4.0)	333 (33)
Tours (1976–1993)	701	35 ± 6 (31–39)	691 (99)	4.2 ± 3.8 (3.0–4.0)	701 (100)
		<0.0001[a]		<0.0001[a]	

[a]*P* value after comparison for age and duration of sexual abstinence (after reciprocal transformation) by analysis of variance. For both variables, the test of Welch (1938) was used since the variances between centres were unequal (Levene's test, 1960), see text.

of the cells, the spermatozoa were counted in the two chambers of the haemocytometer using phase-contrast optics at ×200 magnification. The semen sample was not diluted before counting in the laboratory using the Makler chamber. The percentage of motile spermatozoa was determined in seven laboratories from a calibrated drop of gently mixed semen placed between a glass slide and a coverslip of a known size giving a depth of about 20–25 mm. Sperm motility was analysed at 37°C in four laboratories and at room temperature elsewhere. The percentage of motile spermatozoa was evaluated in all the laboratories at ×100 and ×400 magnification with phase-contrast optics in four to six fields chosen at random. The percentage of motile spermatozoa was taken as the proportion of progressive spermatozoa moving either slowly or rapidly (equivalent to WHO grades 'a' + 'b'; World Health Organization, 1992) relative to the total number of spermatozoa.

Statistical methods

The results were collected in standard microcomputer spreadsheets at each centre. They were recorded in a single file and analysed using the BMDP statistical software (Dixon, 1988) at CECOS Paris-Cochin. The semen characteristics studied were not normally distributed. The BMDP 7D program enabled selection of the appropriate transformation to normalize the distributions and to stabilize the variances of semen characteristics. The optimal transformations in this study were the logarithmic (base 10) transformation for seminal volume, the square root transformation for sperm concentration and total number of spermatozoa and the square transformation for the percentage of motile spermatozoa. An analysis of variance was then performed using transformed data to analyse the differences in semen characteristics between laboratories. The test developed by Welch (1938) was adopted since the variances of all transformed semen characteristics from different centres were unequal (Levene's test; Levene, 1960). The possible confounding effects of the year of semen collection, the man's age and duration of sexual abstinence (Auger *et al.*, 1995) were examined. Variability was small between centres and within a given centre for the man's age but much higher for duration of sexual abstinence (Table I). Moreover, departure from normality was observed for this last variable which was consequently transformed (reciprocal transformation) using the BMDP 7D program before comparison between different centres by analysis of variance. Significant differences were found for age and transformed duration of sexual abstinence between laboratories (Table I). Therefore a multiple regression analysis was performed accounting for these factors together with the possible effect of the year of semen collection and the centres under study. The centres, which are qualitative variables, were introduced into the multiple regression model as 'dummy variables' (Zar, 1984). This enabled the differences between each centre and a reference centre (Paris was chosen arbitrarily) to be analysed rather than an overall difference between centres. Unfortunately, information on duration of sexual abstinence was missing for 29% of the men (Table I). Consequently, the analysis was restricted to a subgroup of 3306 men for whom all the information on the variables entered in the multiple regression model was available. Moreover, the slopes of the semen characteristics plotted against age and duration of sexual abstinence were determined for this subgroup of men using multiple regression analysis. Each individual value was then adjusted to the median age (34 years) and the median period of sexual abstinence before semen collection (3 days) according to the calculated slopes which were not the same in all centres; they depended on noticeable differences between centres in the range of ages and sexual abstinence periods (data not shown). While this procedure did not account for the effect of the year of collection and the differences of slopes for age and duration of sexual abstinence in the different centres, it provided similar results to the multiple regression analysis. Therefore, the mean, median, 25th and 75th centile and 5th and 95th centile of back-transformed semen characteristics adjusted for age and duration of sexual abstinence are presented for each centre. Minimum significance level throughout the study is $P = 0.10$.

Results

The population of 4710 unselected fertile men had a median semen volume of 3.4 ml, a median sperm concentration of 80×10^6 spermatozoa/ml, a median total number of spermatozoa of 264×10^6 in the ejaculate and a median percentage of motile spermatozoa of 65%. Mean and median values of semen characteristics for each centre are presented in Table II. There were significant regional differences for all semen characteristics (all $P < 0.0001$) (Table II). In the regression analysis, the year of semen collection, age and duration of sexual abstinence were correlated with semen characteristics and, compared to Paris, semen characteristics were significantly higher or lower in most of the other areas (Table III). The highest total number of spermatozoa (reflecting the sperm production) was found in the north of France (Lille) and

Table II. Mean values ± SD (median values) of semen characteristics of fertile men from eight regions of France

Region	Seminal volume (ml)	Sperm concentration ($\times 10^6$/ml)	Total sperm count ($\times 10^6$)	Motile spermatozoa (%)
All regions	3.7 ± 1.8 (3.4)	95 ± 70 (80)	337 ± 297 (264)	64 ± 14 (65)
Paris	3.8 ± 1.8 (3.5)	99 ± 73 (81)	360 ± 312 (281)	66 ± 12 (65)[a]
Caen	4.1 ± 1.9 (3.7)	102 ± 86 (78)	409 ± 380 (311)	65 ± 13 (65)
Grenoble	3.7 ± 1.9 (3.5)	82 ± 63 (64)	306 ± 284 (221)	63 ± 17 (70)
Toulouse	3.6 ± 1.7 (3.5)	85 ± 69 (65)	284 ± 262 (211)	66 ± 14 (70)
Bordeaux	3.4 ± 1.8 (3.0)	88 ± 52 (80)	302 ± 253 (249)	69 ± 12 (70)[a]
Lille	3.8 ± 2.0 (3.5)	93 ± 87 (70)	346 ± 392 (230)	67 ± 12 (70)
Rennes	3.2 ± 1.5 (3.0)	99 ± 55 (90)	313 ± 224 (267)	62 ± 13 (60)[a]
Tours	3.9 ± 1.8 (3.6)	95 ± 78 (81)	356 ± 297 (292)	58 ± 16 (60)[a]
	<0.0001[b]	<0.0001[b]	<0.0001[b]	<0.0001[b]

[a]The percentage of motile spermatozoa was evaluated at 37°C in these centres; otherwise it was evaluated at ambient temperature.
[b]*P* value after comparison of transformed semen characteristics (see text) by analysis of variance. For all semen characteristics, the test of Welch (1938) was applied since the variances between centres were unequal (Levene's test, 1960), see text.

Table III. Effects of the study centre, year of semen collection, age and duration of sexual abstinence on semen characteristics from 3306 fertile men studied by multiple regression analysis

Semen characteristic and factors	Regression coefficient	*P*-value	Semen characteristic and factors	Regression coefficient	*P*-value
Seminal volume[b]			Sperm concentration[b]		
Caen[a]	0.081	<0.001	Lille[a]	0.887	<0.001
Lille[a]	0.037	<0.01	Rennes[a]	0.810	<0.001
Tours[a]	0.019	<0.10	Grenoble[a]	–0.391	<0.10
Bordeaux[a]	–0.031	<0.05	Year	–0.036	<0.01
Rennes[a]	–0.047	<0.001	Sexual abstinence[b]	–10.949	<0.001
Toulouse[a]	–0.056	<0.01			
Year	–0.002	<0.05			
Age	–0.002	<0.005			
Sexual abstinence[b]	–0.389	<0.001			
Total number of spermatozoa[b]			Sperm motility [b]		
Lille[a]	2.205	<0.001	Bordeaux[a]	959	<0.001
Caen[a]	1.500	<0.05	Lille[a]	517	<0.001
Toulouse[a]	–1.232	<0.05	Caen[a]	272	<0.10
Year	–0.106	<0.001	Tours[a]	–604	<0.001
Age	–0.056	<0.005	Year	–106	<0.001
Sexual abstinence[b]	–26.538	<0.001	Age	–27	<0.001
			Sexual abstinence[b]	772	<0.05

[a]The regression coefficients are indicated lower (–) or higher (+) for values of the given semen characteristic relative to that recorded in Paris; see text.
[b]Data were introduced into the model after appropriate transformation, see text.

the lowest in the south (Toulouse). The values of semen characteristics when only adjusted for median age and median duration of sexual abstinence (Table IV and Figure 1) were similar to those observed by multiple regression analysis. The highest value for median seminal volume (Figure 1a) was in Caen (4.0 ml), the lowest in Rennes (3.0 ml). The highest values for both mean and median total sperm count were observed in Lille (398 and 287×10^6 respectively) and Caen (364 and 298×10^6 respectively), the lowest in Toulouse (259 and 238×10^6 respectively) and Grenoble (304 and 234×10^6 spermatozoa, respectively). The centres in southern France had the lowest values for mean and median number of spermatozoa in the ejaculate. The highest value for the median percentage of motile spermatozoa was observed in Bordeaux (71%) and the lowest in Tours (59%).

Discussion

The semen collected from unselected fertile volunteers displayed different characteristics depending on the geographical area from which it was obtained. This result remained consistent even after taking into account variations due to the year of semen collection, the subject's age and the duration of sexual abstinence before semen collection in multiple regression analysis. There is currently no clear explanation for such regional differences. Nevertheless, it is important to evaluate the role of factors which may have influenced our results.

Table IV. Mean values ± SD (median values shown in parentheses) of semen characteristics of fertile men from eight regions of France, adjusted for median age (34 years old) and median duration of sexual abstinence (3 days)

Region	Seminal volume[a] (ml)	Sperm concentration ($\times 10^6$/ml)	Total sperm count[a] ($\times 10^6$)	Motile spermatozoa[a] (%)
All regions	3.7 ± 1.8 (3.4)	92 ± 69 (78)	330 ± 286 (263)	64 ± 13 (66)
Paris	3.7 ± 1.7 (3.4)	92 ± 70 (76)	327 ± 281 (257)	65 ± 12 (66)
Caen	4.3 ± 1.8 (4.0)	90 ± 79 (73)	364 ± 267 (298)	65 ± 11 (67)
Grenoble	3.7 ± 1.9 (3.4)	82 ± 60 (65)	304 ± 266 (234)	64 ± 15 (69)
Toulouse	3.2 ± 1.7 (3.1)	86 ± 65 (75)	259 ± 183 (238)	62 ± 13 (64)
Bordeaux	3.4 ± 1.8 (3.1)	90 ± 52 (80)	305 ± 254 (253)	69 ± 12 (71)
Lille	4.1 ± 2.2 (3.7)	103 ± 82 (82)	398 ± 376 (287)	67 ± 12 (69)
Rennes	3.2 ± 1.5 (3.0)	99 ± 54 (90)	317 ± 237 (275)	62 ± 14 (64)
Tours	3.9 ± 1.8 (3.6)	91 ± 77 (73)	337 ± 294 (268)	59 ± 15 (59)

[a]Back-transformed data are presented, see text.

Selection bias

In most studies on human semen characteristics, the populations under study are insufficiently defined. In this study, the men were homogeneous according to their fertility status and mode of recruitment. They had all fathered at least one child and were healthy, unpaid volunteers. Only the first ejaculate was analysed to avoid selection due to semen characteristics. Nevertheless, the men in this study do not exactly represent the French population. They were mainly recruited from urban areas and their socio-professional profile was shifted towards the more educated classes, a common characteristic of semen donors (Auger *et al.*, 1995). Nevertheless, the homogeneity of the population in this study allowed comparisons to be made between centres.

Confounding bias

Two important factors influencing semen characteristics, age and the duration of sexual abstinence before semen collection, were found to differ between centres. A clear distinction in the quality of semen from different centres was evident after the adjustment of data to account for both factors (compare Tables II and IV). The mean total number of spermatozoa in the ejaculate was lowered by ~10% in Paris, Caen and Toulouse while in the latter, the median increased. It was lowered by about 5% in Tours. It was unchanged in Grenoble, Bordeaux and Rennes and it was increased by about 10% in Lille. This clearly illustrates the importance of considering age and duration of sexual abstinence when comparing semen quality data. After adjustment for age and duration of sexual abstinence, the range of variation of mean and median semen characteristics between centres was lowered. Multiple regression analysis revealed significant effects of the three factors: year of collection, age and duration of sexual abstinence. It should be noted that there was a significant decline in the semen characteristics recorded according to the year of semen collection which requires further investigation. Finally, when accounting for these factors in the multiple regression model, variations in all semen characteristics were found between different centres.

Measurement bias

A recent report on external quality control has shown noticeable variations in the determination of semen characteristics between laboratories related to differences in technique (Neuwinger *et al.*, 1990) and it is important to account for this in studies on changes of semen characteristics over time (Bujan *et al.*, 1996; Irvine *et al.*, 1996; Vierula *et al.*, 1996; Van Waeleghem *et al.*, 1996). By contrast, we believe that measurement bias in our study is reasonably low, since data collected in different laboratories over several years were amalgamated for analysis. However, there were small differences in some analysis steps suggesting possible measurement bias. For example, the use of the Makler chamber may overestimate sperm concentration (Ginsburg and Armant, 1990) and the highest median sperm concentration was observed in the centre which used this device (Rennes). In contrast, most of the highest values for motility were observed in centres where the analysis was performed at ambient temperature and not at 37°C (Table II). The evaluation of the percentage of motile spermatozoa is subjective by nature and a higher between-centre coefficient of variation was reported for this characteristic than for sperm concentration (Neuwinger *et al.*, 1990).

Other factors

Natural and man-made factors may have an impact on semen quality. Climatic, topographical, geochemical, physical and biological factors that constitute the natural background of a population vary from one region to another. It is also obvious that cultural factors including lifestyle and psychological pressure (e.g. stress) differ between study centres. The possible influence of stress on human sperm motility has been discussed recently (Fukuda *et al.*, 1996). Previous studies on wildlife and human epidemiology have suggested that environmental factors are involved in the origin of male reproductive disorders (Danish Environmental Protection Agency, 1995) and could be responsible for regional differences as well as secular modifications of semen quality (Auger *et al.*, 1995; Danish Environmental Protection Agency, 1995; Bujan *et al.*, 1996; Irvine *et al.*, 1996; Van Waeleghem *et al.*, 1996). Many chemical compounds which accumulate in the environment disrupt the endocrine system (Danish Environmental Protection Agency, 1995). Compounds taken by pregnant females with oestrogen-like or anti-androgen activity can have an adverse effect on genital development and testicular function of male

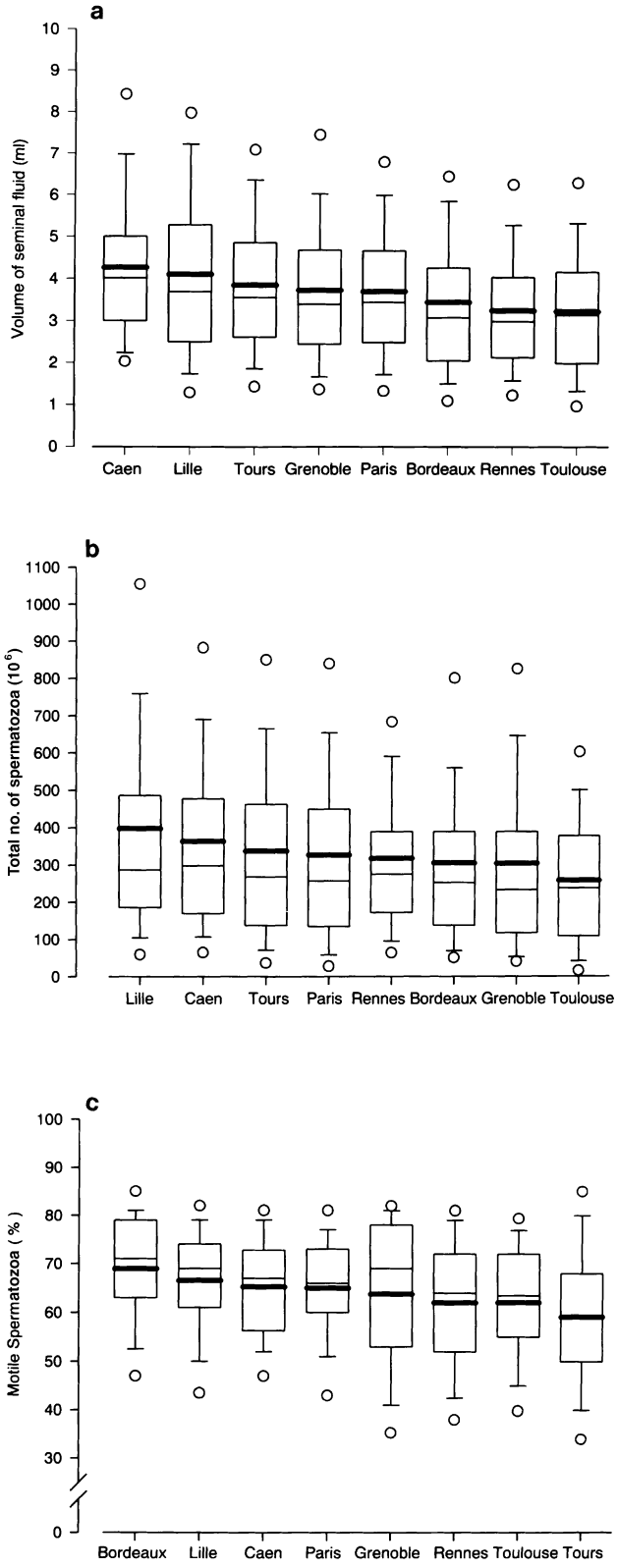

Figure. 1. Box plots of seminal volume, total number of spermatozoa in the ejaculate and percentage of motile sperm of fertile men from eight regions of France. Data were adjusted for median age (34 years old) and median duration of sexual abstinence (3 days). Results plotted: ○, 5th–95th centile; bars, 10th–90th centile; boxes, 25th–75th centile; and median value; **–**, mean value.

offspring (Kelce *et al.*, 1995; Sharpe *et al.*, 1995). Repeated chemical exposure in post-natal life may also have adverse effects on male reproductive health. The potential toxic compounds accumulating in our environment may enter the food chain and the water supply. Differences in sperm counts have been reported reflecting differences in the water supply (Ginsburg *et al.*, 1993). The results reported here may reflect variable environmental factors affecting the inhabitants of different areas. No link has yet been established between the differences we observed in semen quality and the production and/or the distribution of man-made chemical compounds. In Paris and Lille, two major industrial areas, sperm production was in the higher range. The present study also indicated lower sperm production in southern areas of France: Toulouse, Bordeaux and Grenoble. The cause of such observations demands further study. Although the present study was undertaken on unselected semen donors, it would also be interesting to compare semen characteristics of men selected as donors and to detect if similar regional differences in sperm fertilizing ability could be observed through the results of artificial insemination (study in progress).

In conclusion, multidisciplinary studies are needed to determine the possible effects of natural and man-made factors on semen quality. It is also important to verify the present results with prospective studies on semen quality of homogeneous groups of men in different regional or geographical areas. The first step in these studies should be the design and the use of a single standardized method for semen analysis, with appropriate internal and external quality controls.

Acknowledgements

The authors thank Dr Béatrice Ducot and Dr Alfred Spira for valuable and critical discussion of the manuscript, also Jean Claude Juillard for his help in data collection.

References

Adamopoulos, D.A., Pappa, A., Nicopoulou, S. *et al.* (1996) Seminal volume and total number trends in men attending subfertility clinics in the Greater Athens area during the period 1977–1993. *Hum. Reprod.*, **11,** 1936–1941.

Auger, J., Kunstmann, J.M., Czyglik, F. and Jouannet, P. (1995) Decline in semen quality among fertile men in Paris during the past 20 years. *N. Engl. J. Med.*, **332,** 281–285.

Bromwich, P., Cohen, J., Stewart, I. and Walker, A. (1994) Decline in sperm counts: an artefact of changed reference range of 'normal'? *Br. Med. J.*, **309,** 19–22.

Bujan, L., Mansat, A., Pontonnier, F. and Mieusset, R. (1996) Sperm concentration in donors 1977–1992 in Toulouse, France. *Br. Med. J.*, **312,** 471–472.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E. (1992) Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.*, **305,** 609–613.

Danish Environmental Protection Agency (1995) *Male Reproductive Health and Environmental Chemicals with Estrogenic Effects.* Ministry of Environment and Energy, Copenhagen. Miljøprojekt no. 290.

Dixon, W.J. (1988) *BMDP Statistical Software Manual*. University of California Press, Berkeley.

Fisch, H., Goluboff, E.T., Olson, J.H. *et al.* (1996) Semen analysis in 1283 men from the United States over a 25-year period: no decline in quality. *Fertil. Steril.*, **65,** 1009–1014.

Fukuda, M., Fukuda, K., Shimizu, T. *et al.* (1996) Kobe earthquake and reduced sperm motility. *Hum. Reprod.*, **11,** 1244–1246.

Ginsburg, J., Okolo, S., Prelevic, G. and Hardiman, P. (1993) Residence in the London area and sperm density. *Lancet*, **343,** 230.

Ginsburg, K.A. and Armant, D.R. (1990) The influence of chamber characteristics on the reliability of sperm concentration and movement measurements obtained by manual and videomicrographic analysis. *Fertil. Steril.*, **53,** 882–887.

Irvine, D.S., Cawood, E., Richardson, D. *et al.* (1996) Evidence of deteriorating semen quality in the United Kingdom: birth cohort study of 577 men in Scotland over 11 years. *Br. Med. J.*, **312,** 467–470.

Kelce, W.R., Stone, C.R., Laws, S.C. *et al.* (1995) Persistent DDT metabolite p,p′-DDE is a potent androgen receptor antagonist. *Nature*, **375,** 581–585.

Levene, H. (1960) Robust tests for equality of variances. In Olkin, I. (ed.), *Contributions to Probability and Statistics*. Stanford University Press, Palo Alto.

Makler, A. (1980) The improved ten-micrometer chamber for rapid sperm count and motility evaluation. *Fertil. Steril.*, **33,** 337–338.

Neuwinger, J., Behre, H.M. and Nieschlag, E. (1990) External quality control in the andrology laboratory: an experimental multicenter trial. *Fertil. Steril.*, **54,** 308–314.

Olsen, G.W., Bodner, K.M., Ramlow, J..M. *et al.* (1995) Have sperm counts been reduced 50% in 50 years? A statistical model revisited. *Fertil. Steril.*, **63,** 887–893.

Paulsen, C.A., Berman, N.G. and Wang, C. (1996) Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. *Fertil. Steril.*, **65,** 1015–1020.

Sharpe, R.M., Fisher, J.S., Millar, M.M. *et al.* (1995) Gestational and lactational exposure of rats to xenooestrogens results in reduced testicular size and sperm production. *Environ. Health Perspect.*, **103,** 1136–1143.

Van Waeleghem, K., De Clercq, N., Vermeulen, F. *et al.* (1996) Deterioration of sperm quality in young healthy Belgian men. *Hum. Reprod.*, **11,** 325–329.

Vierula, M., Keiski, A., Saaranen, M. *et al.* (1996) High and unchanged sperm counts of Finnish men. *Int. J. Androl.*, **19,** 11–17.

Welch, B.L. (1938) The generalization of Student's problem when several different population variances are involved. *Biometrika*, **34,** 28–35.

World Health Organization (1992) *Laboratory Manual for the Examination of Human Semen and Sperm–Cervical Mucus Interaction.* 3rd edn. Cambridge University Press, Cambridge.

Zar, J.H. (1984) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, pp. 346–347.

Received on October 14, 1996; accepted on January 23, 1997