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Trends in Semen Parameters in the Northeast of Scotland

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ABSTRACT: The data on trends in semen quality are conflicting and sensitive to geographical variations. Although previous British surveys on semen quality indicate a decline, the northeast of Scotland has never been included in these surveys. This is an area with low out migration rates where andrology services for a population of 500 000 are centralized within a single laboratory, thus providing a unique opportunity to study population-based trends in semen quality over time. We investigated trends in semen parameters between 1994–2005, in a cohort of 4832 men attending for routine semen analysis at the Aberdeen Fertility Centre who had a sperm density of greater than 20 million per mL. The main outcome measures were trends in sperm density, sperm motility and motile density in the first semen sample. Linear regression and time series analysis were used to examine trends over time in the semen parameters. The mean and standard deviation (SD) age of all men (n = 5204) in the study was 34 (6) years. The median (inter quartile range) for sperm density and motile density for the study population were 61 (40–91) million/mL and 99 (47–181) millon. The mean (SD) sperm motility was 49 (19)%. Among 4832 men (with sperm count >20 million per mL), data adjusted for age and period of abstinence showed a decreasing trend for sperm density over time, $R^2 = 0.45$ ($P = .017$). There was no such trend in sperm motility and motile density. However, this trend has to be interpreted with caution due to fluctuations in semen parameters, population bias and the retrospective nature of the analysis.

Key words: Fertility, infertility, sperm.

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The last two decades have seen the publication of several reports that suggest a global decline in semen quality (Bostofte et al, 1983; Carlsen et al, 1992; Auger et al, 1995; Irvine et al, 1996; Swan et al, 1997; Bilotta et al, 1999). Within Western Europe, a four-centre study on fertile couples (Jensen et al, 2001) has shown substantial variations in semen parameters. However, the data on trends in semen quality are conflicting and sensitive to geographical variations (Bujan et al, 1996; Fisch et al, 1996a; Benshushan et al, 1997; Costello et al, 2002; Pal et al, 2006). It has also been difficult to prove an association between deterioration in semen quality and male infertility. The quality of existing studies has been questioned on the basis of population and selection bias, variations in laboratory standards, and statistical methodologies used for the analysis. Although previous British surveys on semen quality indicate a decline, the northeast of Scotland has never been included in these surveys. Earlier studies from France indicate variations in different cities within that country (Auger and Jouannet, 1997).

The northeast of Scotland is an area with low outward migration rates (Hall et al, 1989; Batty et al, 2004; General Register Office of Scotland, 2006), where the andrology services for a population of 500 000 are centralized within a single laboratory. This provides a unique opportunity to study population-based trends in semen quality over a period of time. In the present study, we investigated the trends in semen parameters over time in a cohort of men who attended for routine semen analysis with sperm densities greater than 20×10^6/mL.

Materials and Methods

Study Subjects

This was a retrospective, observational study based on data from the Aberdeen Andrology Database. This is a secure database into which data on men who have attended for semen analysis are entered prospectively. The subjects included the male partners of subfertile couples in the Grampian region of Scotland who attended the Aberdeen Fertility Centre between 1994 and 2005. Although initially it appeared that more men attended in the period 2001 to 2005 than during the period 1994 to 1999, further analysis showed no changes in referring

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practice over this period. Oligozoospermic men, azoospermic men, and men with a history of vasectomy were excluded from the current analyses. The etiologies and severities of infertility were not analyzed. In order to minimize selection bias, the first semen sample from each man was used for the present study (by year of donation), and the values of sperm concentration (density), motility, and motile density were analyzed. Data on sperm morphology were excluded due to significant variations in laboratory techniques and criteria for normality over the study period. During the study period, routine semen analysis was performed using the Hamilton Thorne Sperm Motility Analyzer (Beverly, Mass) according to the guidelines of the World Health Organization (WHO). The National External Quality Assessment Service (NEQAS) oversaw the external quality controls for the laboratory four times per year. No nonconformities were reported. Internal audits for adherence to standard operating procedures during sample preparation and analysis, as well as weekly comparisons between technicians were additional internal quality control measures.

Semen Analysis

The laboratory instructed the men to abstain from ejaculation for 3–7 days before providing a sample by masturbation into a sterile plastic container. The sample was allowed to liquefy at 37°C for an initial period of 30–60 minutes, and all analyses were performed within 90 minutes of ejaculation. The volume of the ejaculate was determined by aspirating the liquefied sample into a graduated disposable pipette. To determine the concentration of sperm (10^6/mL), and sperm motility, a 10-μL drop of the semen sample was placed on a commercially produced, fixed-depth capillary fill chamber. The chamber used until 2002 was the Conception Technology Microcell MC-20-2 (San Diego, Calif). This was replaced by the Leja Standard Count 20-μm Analysis Chamber (Nieuw-Vennep, The Netherlands) in the subsequent years. When the chamber was full, the slide was placed in a heated stage at 37°C. The stage was placed on the microscope and the sample was assessed using the Hamilton Thorne HTM-S Semen Analyzer until the year 2000, after which the Hamilton Thorne Version 10 HTM-CEROS was used. A minimum of 200 sperm or 2 frames were counted. Local comparisons of changes in chamber and software were performed. These showed no significant differences with respect to sperm densities or motilities. Progressive motility was determined as the proportion of sperm that showed evidence of movement (WHO grades a and b) to the total number of spermatozoa counted (WHO grades a, b, c, and d) (WHO, 1993, 1999).

Statistical Analysis

The software packages SPSS (13.0) (SPSS Inc, Chicago, Ill) and Microsoft Excel 2000 (Redmond, Wash) were used for statistical analysis. Linear regression and time series analyses were used to examine trends over time in the three semen parameters of interest (ie, density, motility, and motile density).

As all our men were partners in a subfertile relationship, we analyzed the trend in semen parameters for men with a sperm density greater than 20×10^6/mL, to minimize selection bias. In the time trends analysis, only men with a period of abstinence of 1–10 days and who were aged 22–56 years were included, since the consistent linear relationships between mean sperm parameters and these two confounders were no longer evident beyond these limits (Figure 1a and b). Initially, analysis of variance (ANOVA) and Kruskal-Wallis tests were carried out to see if there were any overall differences in sperm density, motility or motile density, for each year of the period 1994 to 2005 (Table 1). Examination of the mean values for the sperm parameters of these men for each year of donation from 1994 to 2005 showed that these values were normally distributed. Thus, log transformation of these data, as previously suggested (Berman et al, 1996), was not required. A linear trend was fitted to the mean of each variable of interest (sperm density, sperm motility, and motile density) across the period of abstinence from 1 to 10 days and across age from 22 to 56 years. The residuals of the fitted linear models were then utilized as the age-adjusted and abstinence-adjusted data for the follow-up period.

Results

Data on semen samples from a total of 6761 men were available for analysis (Figure 2). Of the 6761 men, 5204 men were normal in terms of sperm density (ie, had densities greater than 20×10^6/mL). The raw data on age and sperm motility were normally distributed, while sperm density and motile density were positively skewed. The mean ± SD age of the men (n = 5204) in the present study was 34 ± 6 years. The median (interquartile range) values for sperm density and motile density for the study population were 61 (40–91) $\times 10^6$/mL and 99 (47–181) $\times 10^6$/mL. The mean ± SD for sperm motility was 49 ± 19%.

Since length of abstinence showed strong linear relationships with each of the three sperm parameters (Figure 1a), for the analysis of trend over time we considered the data for men whose period of abstinence was between 1 and 10 days. As shown in Figure 1B, men younger than 22 years and those older than 56 years had large fluctuations in sperm parameters, and since they constituted only a small proportion of the population studied, these men were excluded from any subsequent analysis. Of the 5204 men who had a sperm count of more than 20×10^6/mL, 103 men had incomplete data for age and abstinence, 219 men did not abstain at all or abstained for more than 10 days and 50 men were either younger than 22 years or older than 56 years (Figure 2). These subjects were excluded from the current study, which left 4832 men for analysis. Descriptive statistics for the unadjusted data for each of the sperm parameters for each year from 1994 to 2005 are listed in the Table, along with the results of the ANOVA and Kruskal-Wallis tests. There were signifi-

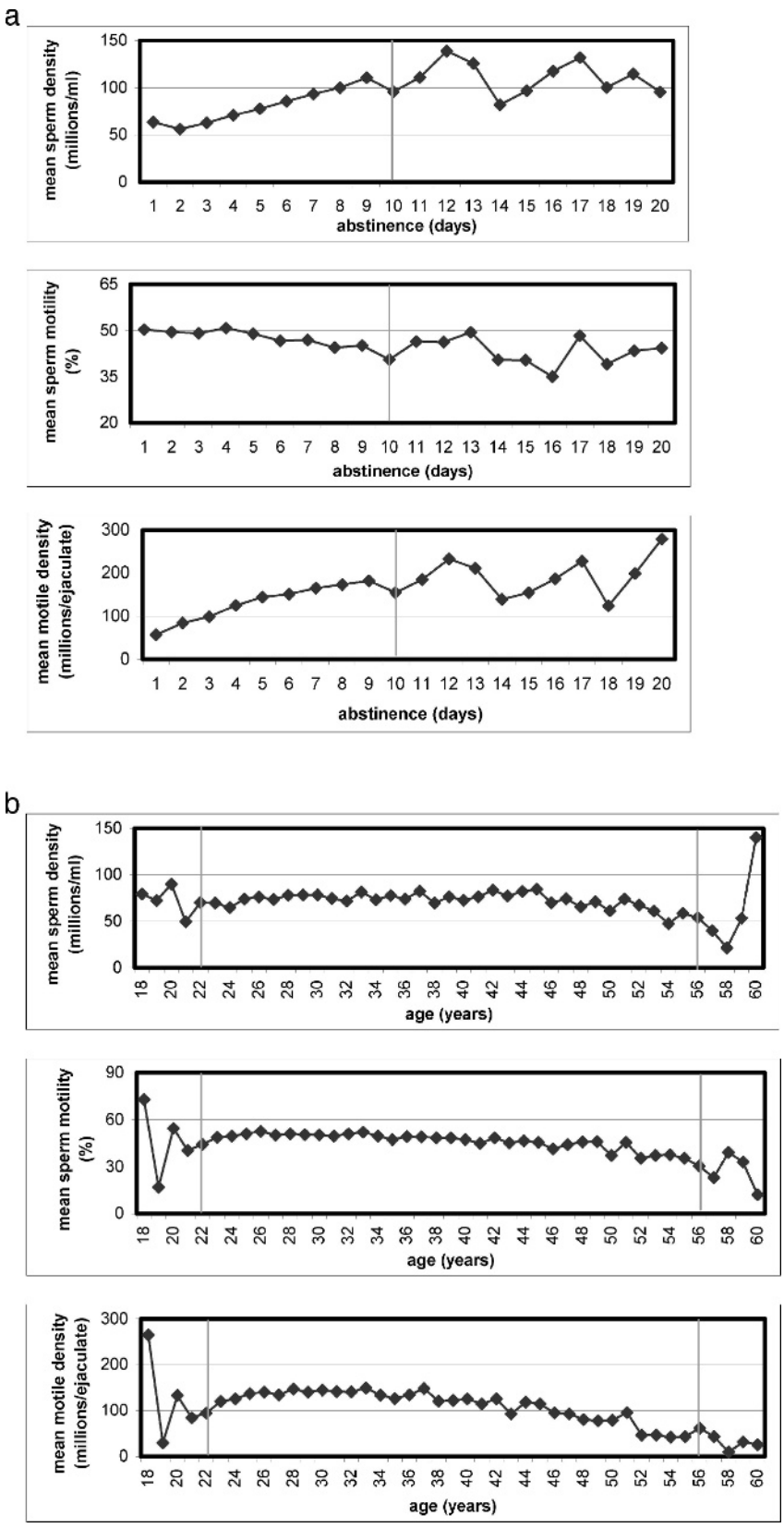

Figure 1. (**a**) Relationship between sperm parameters and period of abstinence. (**b**) Relationship between sperm parameters and age.

*Semen parameters for 4832 men with normal sperm densities**

		Sperm density ($\times 10^6$/mL) Kruskal-Wallis $P < .001$		Sperm motility (%) ANOVA $P = .154$		Motile density ($\times 10^6$ per ejaculate) Kruskal-Wallis $P < .001$	
Year	n	Median	IQR	Mean	SD	Median	IQR
1994	301	75.0	49.0, 118.0	51.5	16.7	118.8	62.9, 215.6
1995	323	61.4	38.7, 98.5	48.5	18.4	97.0	43.7, 209.8
1996	357	76.0	42.4, 115.0	47.5	18.3	110.1	50.6, 193.8
1997	354	66.5	41.8, 95.6	48.6	19.2	112.6	47.6, 190.8
1998	351	56.0	36.4, 85.5	50.0	19.2	102.6	50.8, 170.6
1999	375	71.0	45.7, 107.3	47.5	19.4	119.7	53.0, 241.1
2000	379	61.1	38.3, 92.4	49.0	20.6	96.1	44.4, 194.2
2001	350	53.0	36.4, 78.2	50.0	19.2	90.8	42.3, 170.1
2002	387	56.4	40.2, 80.0	50.4	17.5	91.2	47.7, 155.6
2003	635	57.9	38.0, 81.6	49.6	18.6	92.4	45.8, 166.5
2004	536	57.7	39.3, 79.2	49.0	17.8	89.6	45.6, 159.3
2005	484	55.9	38.9, 80.8	48.4	18.1	87.9	39.0, 153.7

* Abbreviations used: IQR, interquartile range; SD, standard deviation.

cant differences in the averaged annual sperm density and motile density values across the 12-year period.

For the linear regression analysis, the data were adjusted for age and abstinence by first considering the residuals of the fitted linear model, which included abstinence, and second, utilizing these data for fitting a subsequent linear model for age. The residuals of the latter fitted models were then considered as being adjusted for age and abstinence. Figure 3 illustrates the variations in mean sperm density, motility, and motile density in 4832 men with sperm count of more than 20 $\times$ 10^6/mL, across the 12-year period between 1994 and 2005. The unadjusted and adjusted means for each year for each variable were calculated. As shown in Figure 3, decreasing trends over time were noted for sperm density and motile density before the data were adjusted for age and abstinence. The adjusted data show decreased sperm density ($R^2 = 0.45$, $P = .017$), while there was no evidence for similar trends in sperm motility and motile density. There was no significant difference in the mean period of abstinence over this time period.

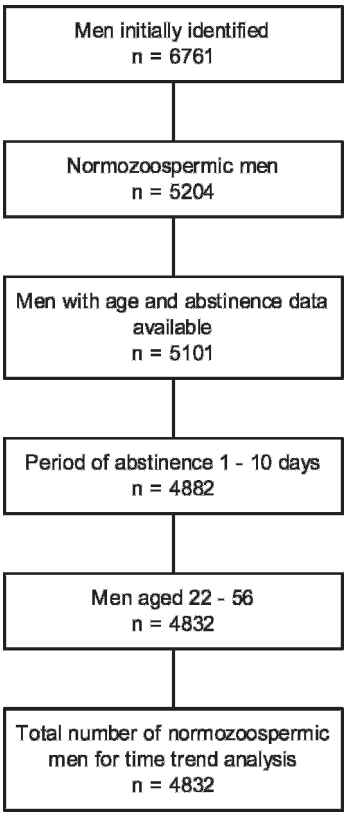

Figure 2. Characteristics of the men included in the analysis.

Discussion

Our results suggest a decline in sperm counts over a 12-year period among men with a sperm density greater than 20 $\times$ 10^6/mL in the northeast of Scotland. Similar trends in sperm motility and motile density values were not observed.

The relatively stable population, large sample size, and the use of a single laboratory to process all the samples are the main strengths of the present study. The availability of data regarding age and abstinence allowed adjustment for these important confounders. One of the main weaknesses of the present study is that all the subjects were men from subfertile partnerships. However, to minimize selection bias, we analyzed the trends only among those men who had a normal sperm density.

Studies on semen parameters should acknowledge the large variation in samples from the same individual and

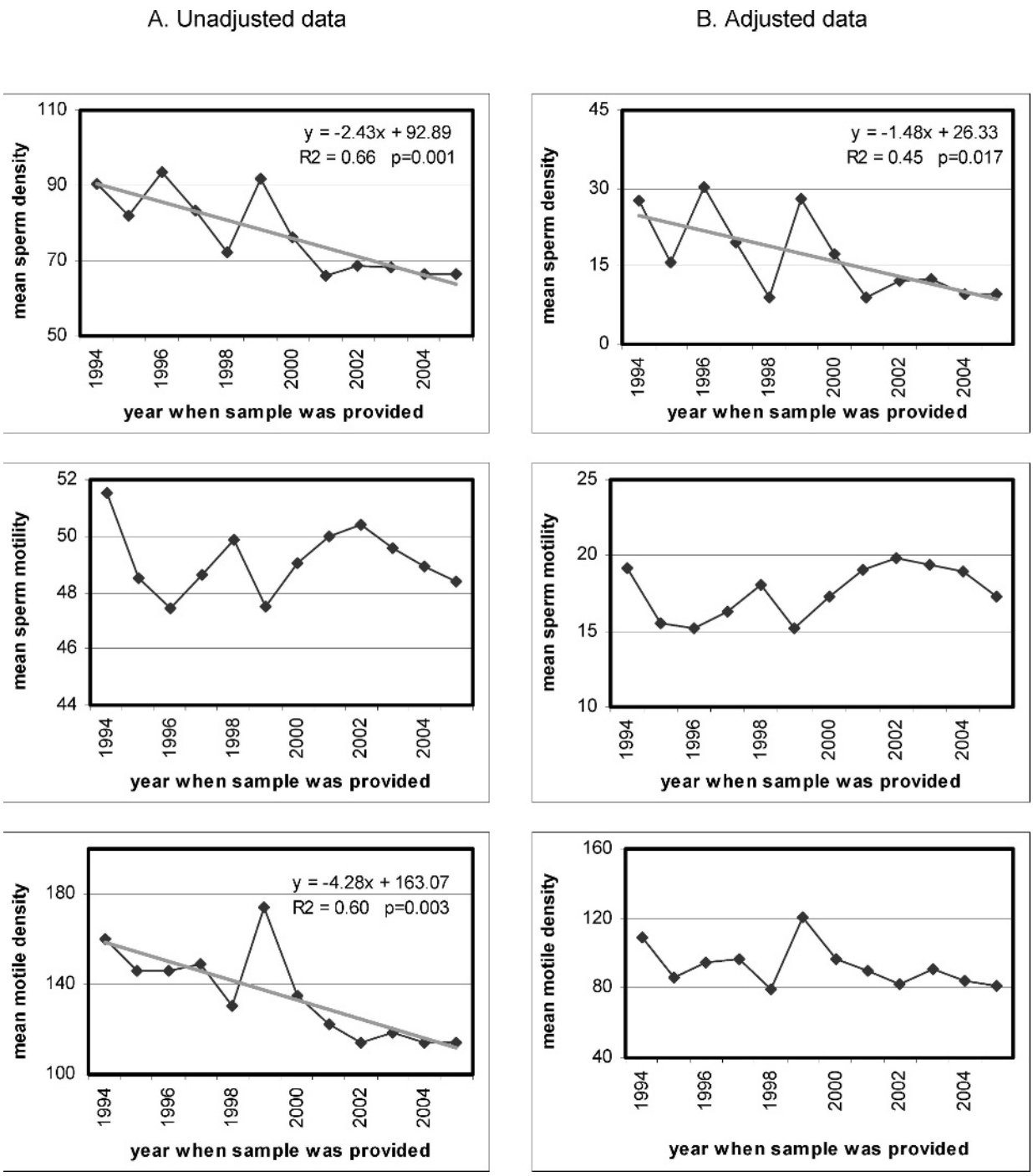

Figure 3. Variations over time in sperm parameters in normozoospermic men. (**A**) Unadjusted data (data before adjusting for age and abstinence). (**B**) Adjusted data (data after adjusting for age and abstinence).

between individuals (Heuchel et al, 1983; Gyllenborg et al, 1999). In order to minimize intraindividual bias, we used the first semen sample from each man. Circannual variation is a controversial topic (Tjoa et al, 1982; Carlsen et al, 2004; Malm et al, 2004). However, as our data were collected over all the 12 months in each year, the impact of seasonal variation was minimized by using the mean annual values. The reasons for fluctuations in semen parameters for men of less than 22 years of age and more than 56 years of age (Figure 1b) are unclear, although these fluctuations may be due to the lower numbers of men in these age groups.

While most studies (Auger et al, 1995; Irvine et al, 1996) have used the conventional method for semen analysis, in the present study, the samples were analyzed using the Hamilton Thorne Method, which gives higher values for sperm density (Neuwinger et al, 1990) and lower values for sperm motility (Sukcharoen and Aribarg, 1995), Therefore, methodological differences do not explain the decreasing trend seen in the present study, as the use of computer-assisted semen analysis has the opposite effect.

This study has the largest sample size of any reported from the United Kingdom. While previous studies have used linear regression analyses, some have taken no account of confounders, such as age and abstinence. Although we considered using multiple linear regression, examination of the residuals from these models indicated high correlation levels, which violated the assumption of independent residuals. Therefore, we used the time series approach. Our study was population-based and adjusted for the confounders of age and abstinence. Our results are in line with those of an earlier study from the United Kingdom (Irvine et al, 1996). Geographical factors may well be responsible for the conflicting results reported in many other studies (Bujan et al, 1996; Fisch et al, 1996a; Benshushan et al, 1997; Costello et al, 2002; Pal et al, 2006).

In the present study, the sperm densities for subfertile men were lower than those reported in Danish (Rasmussen et al, 1997), French (Auger et al, 1995), and Finnish (Vierula et al, 1996) studies but were similar to the mean sperm concentrations in the preliminary unpublished results of the CHAPS-UK study. This suggests that British men have a lower average sperm concentration than men in most parts of Europe. There is a wide geographical variation in the mean levels of semen parameters. A Danish study (Rasmussen et al, 1997) from a similar subfertile population during the same time interval reported a high mean sperm density of 183.7×10^6/mL, while the mean sperm concentration of subfertile men in Korea was low at 60.5×10^6/mL. It is of note that the mean sperm density for all 1055 men from subfertile partnerships in the Danish study (183.7×10^6/mL) was well above the maximum sperm density (98×10^6/mL) in the Scottish study (Irvine et al, 1996) of fertile men who were semen donors for the Gamete Biology Research study.

Fisch et al (1996b) have highlighted the variation in mean sperm concentrations in fertile men in different countries. Within the United States, the mean concentrations of sperm in fertile populations were as high as 134×10^6/mL in New York and as low as 48×10^6/mL in Iowa (Fisch et al, 1996b). In addition, the reported mean sperm concentrations in developing countries have been reported as being low (Aribarg et al, 1986; Osegbe et al, 1986).

Various explanations have been put forward for the observed decline in sperm counts. General lifestyle factors, such as smoking (Storgaard et al, 2003), alcohol, drug use, and obesity may have etiological roles. In Scotland (Boyle et al, 1987), as in many other parts of the world (Bergstrom et al, 1996; dos Santos Silva et al, 1999), the incidence of testicular cancer, particularly in the age group of 15–44 years, has increased by about 50%. There have also been increases in other male genital abnormalities, such as cryptorchidism and hypospadias, in many populations. This highlights the probable role of intrauterine etiological mechanisms. Endocrine-disrupting chemicals may play a causal role (Murray et al, 2001). Exposure to estrogens during fetal life has been implicated (Storgaard et al, 2006). However, the much higher estrogen levels noted in twin pregnancies, despite these being more potent forms of estrogen, do not appear to result in lower sperm counts in adulthood (Storgaard et al, 2002). Some of the studies reporting declines in sperm counts have also noticed qualitative differences in semen parameters. Whether this is due to infections, such as with *Chlamydia epididymoorchitis*, remains to be investigated (Eley et al, 2005). The current literature highlights the increasing prevalence of genital infections among men and women (LaMontagne et al, 2004). Many factors may be operational, and it was not possible to assess the effects of causative factors in the present study.

Although there is some suggestion that sperm concentrations may be decreasing in some parts of the world, there is no conclusive evidence of decreased fertility potential in the human male population. It may be that semen parameters are poor predictive indicators of fertility potential. Alternatively, undetected compensatory mechanisms may be functioning.

We have described how Scottish men who attended a fertility clinic show a decline in semen density over a 12-year period. However, this trend has to be interpreted with caution due to fluctuations in semen parameters, population bias, and the retrospective nature of the analysis. More prospective studies are required to investigate this phenomenon and to investigate the roles of contributory factors.

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