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Original Article

Single semen analysis as a predictor of semen quality: clinical and epidemiological implications

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Abstract

It is generally thought that a single ejaculate is a bad predictor of semen quality of a subject, because of significant intra-individual variation. Therefore, we investigated the degree to which the results of a first semen analysis differ from that of a second analysis among men from a general population in Norway. In addition, we analysed how the two different semen results mirrored the overall semen quality assessment. A total of 199 volunteers participated in the study and delivered two semen samples with an interval of 6 months. The semen parameters were determined according to the World Health Organization (WHO) 1999 guidelines, which were also used to determine whether semen quality was normal or abnormal. In addition, the DNA fragmentation index (DFI) was determined using the Sperm Chromatin Structure Assay. The two samples from each individual were very similar with regard to standard semen parameters and DFI ($r_{s:}$ 0.67–0.72), and there were no significant systematic differences between the two samples. The result of the first sample (normal/abnormal) was highly predictive of the overall conclusion based on the two samples (sperm concentration: in 93% of the cases (95% confidence interval [CI]: 89%–96%); sperm motility: in 85% of the cases (95% CI: 79%–89%); overall semen quality: in 85% of the cases (95% CI: 80%–90%). In epidemiological studies, one ejaculate is a sufficient indicator of semen quality in a group of subjects. In a clinical situation, when the question is whether the semen quality is normal or not, the first ejaculate will, in at least 85% of cases, give a correct overall conclusion.

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1 Introduction

Semen analysis is still the cornerstone for the evaluation of infertility in men [1]. Moreover, the assessment of semen characteristics is widely used in toxicological, occupational and environmental studies that address the issue of different types of exposure within

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these research fields in relation to male reproductive function [2]. However, in clinical and epidemiological studies, the use of conventional semen parameters, such as sperm concentration, motility and morphology, as markers of male reproductive function has been questioned because of a proposed intra-individual variation in semen characteristics [3–7].

The need for multiple semen analyses during evaluation of the semen quality of a subject has been stressed in several reports [7–9]. However, a significant proportion of these studies have been based on relatively few subjects. Moreover, in most cases, the results were expressed only in terms of coefficient of variation (CV) and were not considered in light of clinical practice, wherein semen parameters are evaluated in relation to the standard WHO criteria and categorized as either 'normal' or 'abnormal' [10]. In this context, some level of intra-individual variation may have a relatively low impact on the clinical decision regarding further investigation and/or treatment offered to the couple. The issue of normal or abnormal semen quality is only one of the factors that must be considered. With regard to epidemiological studies, there is a lack of proof that the intra-individual variation in semen parameters has any significant impact when comparing two groups of subjects, or when conducting a longitudinal follow-up study of one group of individuals [11]. Furthermore, the integrity of the sperm DNA has received an increasing amount of attention as a new marker for semen quality. However, there is only limited knowledge about the day-to-day variation of this parameter. We report, in a recent study of men from infertile couples, that the level of intra-individual variation for the DNA Fragmentation Index (DFI), as measured by the sperm chromatin structure assay (SCSA), was the same as that for the standard sperm parameters, and the CV was 29% [12]. However, the level of variation in a group of men who do not have infertility problems is not known.

As part of the study addressing the issue of seasonal variation in semen parameters, a group of ~100 Norwegian men living south of the Arctic Circle and 100 men living north of the Arctic Circle delivered two semen samples with an interval of ~6 months; no seasonal variation in semen parameters in either location was reported [13]. These results gave us an opportunity to study the impact of intra-individual variation in semen parameters, including DFI, in a clinical and epidemiological setting. The aim of the present study was to investigate whether the proper evaluation of semen quality in a group of subjects is dependent on the use of multiple semen samples. We also determined the agreement between the two samples in an attempt to evaluate to which degree a single ejaculate could be used for classifying semen quality of a subject in relation to the WHO criteria for semen quality. In addition, the importance of standardization of the abstinence time was evaluated based on the question, 'did similar abstinence times at the two sample occasions from each individual increase the agreement?'

2 Materials and methods

2.1 Subjects

The study was advertised on the radio and in local newspapers. Men who fulfilled the following criteria were recruited: (a) the participants had to be between 19 and 40 years old; (b) the participants had to have lived in either one of the two Norwegian towns of Tromsoe or Oslo for a minimum of 1 year before the study commenced; and (c) the participants were to remain in the area during the time of the study. A total of 207 men were recruited, and among them 199 delivered two semen samples. The median age of the men was 29 years (5th and 95th percentiles: 21–38 years). The time between the first and the second sample was 6 months. All subjects provided a written consent. The subjects received 300 NOK (~37 EURO) after delivering the first semen sample and 700 NOK after delivering the second semen sample. A more detailed description of the participants and the sampling periods can be found elsewhere [13]. The study was approved by the Ethical Committee of Lund University, Sweden, and the Regional Committee for Medical Research Ethics, Southern Norway.

2.2 Semen analysis

All of the men were asked to abstain from sexual intercourse and from ejaculating (that is, sexual abstinence) for 2–3 days before their delivering the semen sample. In each case, the length of the abstinence period was recorded. On the first and second sampling occasions, 60% and 58% of men had a sexual abstinence for a period of 2–3 days, respectively. The background characteristics regarding abstinence time and semen parameters are given in Table 1. The semen samples were collected by masturbation and delivered to the laboratory within 60 min. The semen analysis was done according to the WHO recommendations, and

Table 1. Abstinence time and sperm parameters sampled on two occasions among 201 Norwegian men.

Variable	Mean	SD	Median	Fifth, ninety-fifth percentile
Abstinence time (h)				
First sample	85	48	72	48, 168
Second sample	80	36	72	36, 168
Semen volume (mL)				
First sample	3.8	1.6	3.8	1.4, 6.8
Second sample	3.8	1.6	3.6	1.4, 7.0
Mean from both samples	3.8	1.5	3.7	1.6, 6.6
Sperm concentration ($\times 10^6$ per mL)				
First sample	70	59	57	7, 184
Second sample	68	48	60	10, 181
Mean from both samples	69	49	60	8, 164
Total sperm count ($\times 10^6$)				
First sample	261	229	205	20, 707
Second sample	254	211	205	26, 670
Mean from both samples	258	190	202	23, 612
Sperm motility a+b (%)[a]				
First sample	46	11	47	24, 60
Second sample	46	9.9	48	28, 59
Mean from both samples	46	9.3	48	28, 58
DNA fragmentation index (%)				
First sample	12	8.0	9.7	4.0, 29
Second sample	11	6.7	9.2	4.3, 25
Mean from both samples	12	6.9	9.9	4.3, 22

[a]World Health Organization 1999.

the semen volume was estimated by weighing the container with and without the sample [10]. After liquefaction at 37ºC, and within 1 hour of ejaculation, the samples were analysed for sperm motility (graded: a (rapidly progressive), b (slowly progressive), c (non-progressive); or d (immotile)). Total progressive motility was defined as a + b sperm motility. The semen volume and the sperm concentration were also assessed. For two men, a motility assessment was not done. Thus, only 197 of the 199 subjects were available for comparison of this parameter. A concentration assessment was performed using positive displacement pipettes and an improved Neubauer chamber.

The semen samples from both locations were analysed by two laboratory technicians. In each case, the same technician examined both samples from a particular subject without knowing the previous result, that is, the technician examined the second sample without knowing the result of the first sample. The inter-observer CV was 9% for the sperm concentration and 5% for the motility assessment.

2.3 SCSA analysis

After liquefaction at 37ºC and within 1 h of ejaculation, 200 μL of the ejaculate was stored at –80ºC for the subsequent SCSA. The details of this analysis have previously been described [11]. Briefly, the SCSA is based on the phenomenon that chromatin with DNA strand breaks has a tendency to become denatured when exposed to an acid–detergent, whereas normal chromatin remains stable. The SCSA measures the *in situ* denaturability of sperm DNA using acridine orange (AO). AO is a metachromatic dye that differentially stains double- and single-stranded nucleic acids. After blue-light excitation, AO molecules that intercalate into the intact (double-stranded) DNA emit a green fluorescence, whereas AO molecules bound to denatured (single-stranded) DNA emit a red fluorescence. The extent to which the DNA can be denatured is expressed as the DFI, which is the ratio of red to total (red plus green) fluorescence intensity. Thus, the DFI represents the proportion of cells containing denatured DNA.

In total, 5 000 cells were analysed by FACSort

(Becton Dickinson, San Jose, CA, USA).

The DFI was calculated using the List View software (Phoenix Flow Systems, San Diego, CA, USA). An intra-laboratory CV of 4.5% was found after repeated measurements of the same reference sample.

2.4 Statistical analyses

The bivariate correlations between the first and the second sample for the semen variables (semen volume, sperm concentration, total sperm count, sperm motility and DFI) were assessed by Spearman's correlation coefficients (r_s). We also calculated the differences for the semen parameters between the first and the second samples and presented the median difference with fifth and ninety-fifth percentiles. The Wilcoxon paired tests were used to evaluate whether there were systematic differences between the two samples. The statistical significance was defined by $P < 0.05$. In addition, we created two dichotomized variables for the sperm concentration data (cutoff points $< 20 \times 10^6$ and $< 40 \times 10^6$ sperms per mL, respectively), two dichotomized variables for the motility data (cutoff points a < 25% and a + b < 50%, respectively) and the DFI at one cutoff point (< 20%). Abnormal concentration was defined as $< 20 \times 10^6$ sperms per mL, and abnormal motility as rapidly progressive motility with a < 25% and a + b < 50%. Abnormal semen quality was defined as either abnormal sperm concentration or abnormal motility. Kappa statistics were used to evaluate the agreement between the two sampling times for these variables. The Kappa values were interpreted according to the guidelines given by Altman [14], that is, < 0.20: poor; 0.21–0.40: fair; 0.41–0.60: moderate; 0.61–0.80: good; and 0.81–1.00: very good; In addition, we also calculated the clinical predictive values, that is, given normal or abnormal semen quality in the first semen sample, the clinical predictive value measures the probability that the overall conclusion will be the same after evaluation of two samples compared with the first sample only. In this context, the overall semen quality was considered to be normal if either of the two samples was normal with regard to both sperm concentration and motility.

The analyses were performed for all the subjects, that is, irrespective of the lengths of the abstinence time at the two sampling times, as well as separately for the two subgroups in which the individual difference in abstinence time between the two sampling times was 12 h and 24 h.

3 Results

For all of the individuals, the correlations between the two samples were very similar with regard to semen volume, sperm concentration, total sperm count, rapidly progressive motility and DFI (r_s: 0.67–0.72; Figures 1–3; Table 2), although the correlation was somewhat lower for total progressive motility ($r_s = 0.59$). When we analysed the subset of men with a smaller difference in abstinence time, that is, the difference in abstinence time between the two sampling times was 12 h, the correlation for the semen volume increased from 0.71 to 0.84 (Table 2). For the other variables, this tendency was not observed. There was a statistically significant difference ($P = 0.03$ from Wilcoxon's paired test) in the fraction of rapid progressive motile sperm between the two occasions when all of the individuals were included in the analysis. However, this difference almost completely disappeared when we included only the men with a smaller difference in abstinence time (12 h; $P = 0.90$) in the analysis. There were no significant systematic differences between the two occasions for any of the other variables, and this was irrespective of a difference in the abstinence time between the two sample occasions (that is, all $P > 0.05$).

For dichotomized variables, the agreement between the two sample occasions varied between moderate and good for the sperm concentration and rapidly progressive motility (a), and was fair for the total progressive

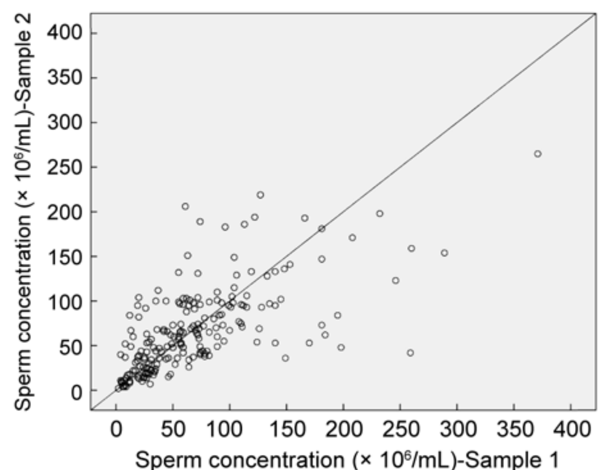

Figure 1. The sperm concentrations in two semen samples delivered after a 6-month interval by volunteers from Oslo and Tromsoe (Norway), r_s (Spearman's correlation coefficients) = 0.70.

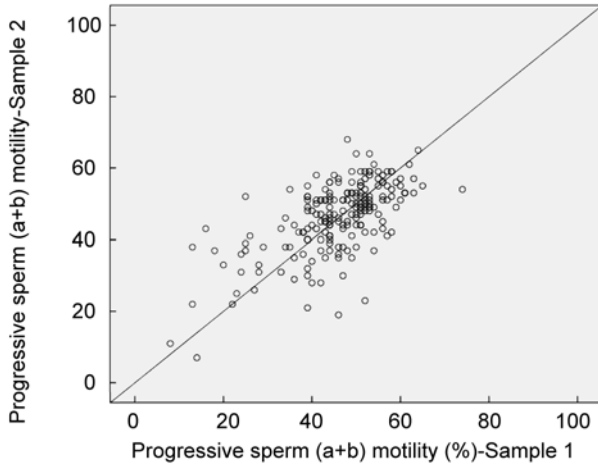

Figure 2. The total progressive sperm motility (a + b) in two semen samples delivered after a 6-month interval by volunteers from Oslo and Tromsoe (Norway), r_s (Spearman's correlation coefficients) = 0.59.

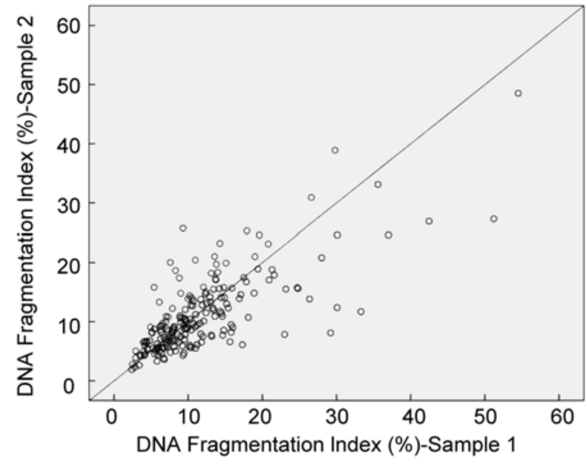

Figure 3. The DNA fragmentation index (DFI) in two semen samples delivered after a 6-month interval by volunteers from Oslo and Tromsoe (Norway), r_s (Spearman's correlation coefficients) = 0.72.

Table 2. Correlations (Spearman's = r_s) between sperm parameters sampled on two occasions among Norwegian men.

Variables	All (n = 199)		Difference in abstinence time: 24 h (n = 140)		Difference in abstinence time: 12 h (n = 88)	
	r_s	Median difference (fifth, ninety-fifth percentile)	r_s	Median difference (fifth, ninety-fifth percentile)	r_s	Median difference (fifth, ninety-fifth percentile)
Semen volume (mL)	0.71	–0.1 (–1.9, 2.0)	0.80	–0.2 (–1.6, 1.8)	0.84	–0.2 (–1.7, 1.5)
Sperm concentration (× 10^6/mL)	0.70	–2.0 (–106, 73)	0.70	–2.0 (–94, 66)	0.72	–3.0 (–70, 72)
Total sperm count (× 10^6 per ejaculate)	0.67	–5.4 (–326, 344)	0.69	–7.4 (–279, 265)	0.72	–11 (–238, 272)
Rapid progressive (a) motility (%)	0.69	1.5 (–12, 16)	0.67	0 (–16, 16)	0.72	0 (–13, 15)
Total progressive (a + b) motility (%)	0.59	1.0 (–14, 14)	0.54	0 (–14, 16)	0.59	0 (–14, 12)
DNA fragmentation index (%)	0.72	–0.2 (–9.2, 7.4)	0.73	–0.4 (–8.9, 5.1)	0.72	–0.3 (–7.6, 4.8)

Median differences based on individual continuous data with the fifth and ninety-fifth percentiles are included. Corresponding data for men whose difference in abstinence time was 24 h and 12 h are also presented.
For all r_s, $P < 0.001$.

motility (a + b) (Table 3). When the sperm motility was categorized as normal or abnormal, the agreement was fair, but borderline moderate, and for the semen quality, the agreement was moderate. For the rapidly progressive motility, abnormal motility and semen quality, the agreement improved when the difference in the abstinence time was reduced. With regard to the DFI, the Kappa values varied between 0.46 and 0.55, that is, a moderate agreement.

If there was an abnormal semen quality in the first sample, there was a 67% (60 out of 89; 95% confidence interval [CI] 57%–77%, Table 3) probability that the second sample was also abnormal. If the first sample was categorized as normal, there was a 76% (82 out of 108; 95% CI: 67%–84%) probability that the second sample was also normal. A similar conclusion was drawn regarding the second sample; in other words, given that the second sample was normal, the probability that the first sample was also normal was 74%. The result of the first sample (normal/abnormal) was predictive for the overall conclusion based on two samples in 93% of the cases (186 out of 199; 95% CI 89%–96%) in terms of the concentration, in 85% of the cases (168 out of 197; 95% CI 79%–89%) in terms of motility and in 85% of the cases (168 out of 197; 95% CI: 80%–90%) in terms of the overall semen quality.

Table 3. Agreement (Kappa values) between sperm concentrations and total progressive motility in semen samples collected on two occasions from Norwegian men.

First semen sample		Second semen sample								
		All			Difference in abstinence time					
					24 h			12 h		
		Yes	No	Kappa	Yes	No	Kappa	Yes	No	Kappa
Sperm concentration										
$< 20 \times 10^6$ per mL	Yes	18	13		13	10		7	5	
	No	13	155	0.50	9	108	0.50	6	68	0.48
$< 40 \times 10^6$ per mL	Yes	51	22		37	17		24	9	
	No	12	114	0.62	12	74	0.56	8	45	0.58
Sperm motility										
a < 25%[a]	Yes	66	35		46	20		28	9	
	No	22	74	0.42	16	57	0.48	11	38	0.53
a + b < 50%[a]	Yes	83	34		58	24		40	12	
	No	27	53	0.37	22	35	0.32	12	22	0.42
Abnormal[b]	Yes	49	31		34	19		22	8	
	No	26	91	0.39	20	66	0.41	12	44	0.50
Semen quality										
Abnormal[c]	Yes	60	29		42	19		25	10	
	No	26	82	0.44	20	58	0.43	11	40	0.50
DNA fragmentation index										
≥ 20%	Yes	10	11		5	5		2	2	
	No	7	161	0.47	5	116	0.46	1	74	0.55

[a]World Health Organization 1999. [b]Abnormal sperm motility defined as a < 25% and a + b < 50%. [c]Abnormal semen quality defined as sperm concentration $< 20 \times 10^6$ sperms per mL or abnormal sperm motility. Corresponding data for men whose difference in abstinence time was 24 h and 12 h are also shown.

These figures were very similar when we investigated the subset of individuals with smaller differences in abstinence time (12 h), and were 94% for concentration, 91% for motility and 88% for the overall semen quality.

4 Discussion

Semen parameters are usually considered to be subject to significant intra-individual variation. For that reason, the value of assessing a single ejaculate within the framework of epidemiological studies has been questioned, and in the clinical setting, at least two [12, 15, 16] or even three [7] semen analyses have been recommended. After an investigation of a group of ~200 volunteers, our data indicate that the correlations between two ejaculates obtained during a 6-month interval were high, and that there were no systematic differences. When the variables were dichotomized, the agreements were fair, in general. In the context of a clinical investigation, the result for the first semen analysis was predictive for the overall evaluation of semen quality based on two ejaculates in ~90% of the cases.

Our conclusions regarding the value of single ejaculates in epidemiological studies are very similar to those made in a recent paper by Stokes-Riner *et al.* [11], concluding that 'As long as the model for semen quality adjusts for important covariates, it makes little difference whether the analysis includes men who give one semen sample or two'. The main difference between their study and our study was that they had an average of 24 days between the first and the second sample, whereas we had 6 months. Another difference is that our study also included information regarding SCSA, whereas the study by Stokes-Riner *et al*. [11] included morphology data. In the study by Stokes-Riner *et al.*, [11] the length of the abstinence period was included in the statistical analysis as one of the covariates. In our study, the difference between the first and second ejaculate was only slightly affected by a reduction in the difference in abstinence time to < 12 h. The relatively low

impact of reducing difference in abstinence time might be due to the fact that 90% of all subjects did have an abstinence period of 2–7 days, as recommended by WHO [10].

When evaluating the results of this study, several sources of bias should be considered. The participants were recruited by advertising in the local media, and thus they might not be representative of the general population. However, the prevalence of infertility problems among the participants [13] was similar to what has been reported for Western countries [17], and the sperm count levels were similar to those for the general Norwegian population [18]. Furthermore, each individual served as his own control. Accordingly, it is unlikely that a selection bias affected the results. When performing an infertility examination, the percentage of men with poor sperm quality will be higher than that of the volunteers included in the current study. However, there is no indication that intra-individual variation in semen parameters is more pronounced among men from infertile couples when compared with healthy controls [7].

Intra- and inter-laboratory variations in the assessment of semen quality are also factors that may influence the results of an assessment of the semen quality. However, in this study, the same laboratory staff and equipment were used in both Tromsoe and Oslo, which minimized the inter-laboratory variation. Furthermore, the same technician examined both the semen samples from each individual.

The current study was originally designed to assess seasonal variation in semen parameters. If any seasonal variation in semen parameters did exist, such a phenomenon would tend to increase the intra-individual variation and thereby lower the agreement between sample one and two. However, even in Tromsoe, wherein the difference between the amount of daylight exposure during the summer and winter is rather extreme, no such variation could be detected [13]. The average time between the two semen samples delivered for this study was 6 months. This implies that, in most subjects, the semen quality will be identically categorized, although a period spanning over as many as three spermatogenic cycles has passed between the sample collections.

It is important to stress that high correlations do not necessarily result in good agreements. For instance, a systematic difference might result in a very high correlation but in a poor agreement. In our analyses, we investigated this by including different statistical analyses, and no such systematic difference was observed.

Our analysis included the most common semen parameters, such as ejaculate volume, concentration and motility, but not morphology. However, there are no published data showing that intra-individual variation in sperm morphology might be more pronounced than in other semen parameters [5–6, 8]. In comparison to the paper by Stokes-Riner *et al.* [11] we also included DFI in the analyses. The conclusion regarding this variable was very similar to that regarding the other semen parameters, which seems to be in agreement with our previous findings made in men who were referred for analysis because of infertility [12]. However, in the present study, it was only a small fraction of men with a DFI above 20%. This cutoff point was selected because it has been shown that *in vivo* fertility decreases when the DFI exceeds this percentage [19].

In conclusion, our study provides further evidence to suggest that, in epidemiological studies, one ejaculate is a sufficient indicator of semen quality. In clinical situations, in which the semen quality is questionable, the first ejaculate, in almost 90% of cases, will give a correct overall conclusion. The semen quality is relatively robust to individual variation in the length of abstinence period, at least as long as it varies in length from 2 to 7 days. To be able to transfer our findings to clinical practice, they need to be confirmed in a group of subjects who have been referred for analysis because of fertility problems. However, our findings suggest that a single ejaculate is in fact a better predictor of overall semen quality than was thought previously. Therefore, one semen analysis might be considered to be sufficient in some groups of infertile couples during the preparation of the future guidelines for an infertility analysis.

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References

1 Rowe PJ, Comhaire F, Hargreave TB, Mahmoud AMA.

WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. 1st edn. United Kingdom: Cambridge University Press; 2000.
2 Bonde JP, Giwercman A, Ernst E. Identifying environmental risk to male reproductive function by occupational sperm studies: logistics and design options. Occup Environ Med 1996; 53: 511–9.
3 Knuth UA, Kuhne J, Bals-Pratsch M, Nieschlag E. Intra-individual variation of sperm velocity, linearity, lateral head displacement and beat frequency in healthy volunteers. Andrologia 1988; 20: 243–8.
4 Mallidis C, Howard EJ, Baker HWG. Variation of semen quality in normal men. Int J Androl 1991; 14: 99–107.
5 Alvarez C, Castilla JA, Martinez L, Ramirez JP, Vergara F, *et al*. Biological variation of seminal parameters in healthy subjects. Hum Reprod 2003; 18: 2082–8.
6 Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. Fertil Steril 2004; 82: 358–66.
7 Keel BA. Within- and between-subject variation in semen parameters in infertile men and normal semen donors. Fertil Steril 2006; 85: 128–34.
8 Oshio S, Ashizawa Y, Yotsukura M, Tohyama Y, Iwabuchi M, *et al*. Individual variation in semen parameters of healthy young volunteers. Arch Androl 2004; 50: 417–25.
9 Poland ML, Moghissi KS, Giblin PT, Ager JW, Olson JM. Variation of semen measures within normal men. Fertil Steril 1985; 44: 396–400.
10 World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge: Cambridge University Press; 1999.
11 Stokes-Riner A, Thurston SW, Brazil C, Guzick D, Liu F, *et al*. One semen sample or two? Insights from a study of fertile men. J Androl 2007; 28: 638–43.
12 Erenpreiss J, Bungum M, Spano M, Elzanaty S, Orbidans J, *et al*. Intra-individual variation in sperm chromatin structure assay parameters in men from infertile couples: clinical implications. Hum Reprod 2006; 21: 2061–4.
13 Malm G, Haugen TB, Henrichsen T, Bjørsvik C, Grotmol T, *et al*. Reproductive function during summer and winter in Norwegian men living north and south of the Arctic circle. J Clin Endocrinol Metab 2004; 89: 4397–402.
14 Altman DG. Practical Statisctics for Medical Research. London (UK): Chapman & Hall; 1991.
15 Berman NG, Wang C, Paulsen CA. Methodological issues in the analysis of human sperm concentration data. J Androl 1996; 17: 68–73.
16 Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, *et al*. Best practice policies for male infertility. Fertil Steril 2002; 77: 873–82.
17 Hull M, Glazener C, Kelly NJ, Conway DI, Foster PA, *et al*. Population study of causes, treatment, and outcome of infertility. Br Med J 1985; 291: 1693–7.
18 Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, *et al*. East-west gradient in semen quality in the Nordic-Baltic area: a study of men from the general population in Denmark, Norway, Estonia and Finland. Hum Reprod 2002; 17: 2199–208.
19 Bungum M, Humaidan P, Axmon A, Spano M, Erenpreiss J, *et al*. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007; 22: 174–9.