Human Reproduction, Vol.24, No.2 pp. 459–469, 2009
Advanced Access publication on December 1, 2008 doi:10.1093/humrep/den399

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Semen quality of 1346 healthy men, results from the Chongqing area of southwest China

Yafei Li[1], Hui Lin[1], Mingfu Ma[3], Lianbing Li[3], Min Cai[3], Niya Zhou[2], Xue Han[2], Huaqiong Bao[3], Liping Huang[4], Caizhong Zhu[1], Chuanhai Li[3], Hao Yang[3], Zhonglin Rao[1], Ying Xiang[1], Zhihong Cui[2], Lin Ao[2], Ziyuan Zhou[2], Hongyan Xiong[1], and Jia Cao[2,5]

[1]Department of Epidemiology, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, People's Republic of China [2]Department of Hygienic Toxicology, College of Preventive Medicine, Third Military Medical University, Chongqing 400038, People's Republic of China [3]Chongqing Institute of Science and Technology for Population and Family Planning, Chongqing 400020, People's Republic of China [4]Chongqing Shapingba Center for Disease Control and Prevention, Chongqing 400038, People's Republic of China

[5]Correspondence address. Tel: +86-023-68752271; Fax: +86-023-68752276; E-mail: caojia@mail.tmmu.com.cn/caojia1962@126.com

TABLE OF CONTENTS

BACKGROUND: Large studies on semen quality of the general healthy population from China are rare.

METHODS: A cross-sectional study was performed to evaluate the semen quality of 1346 healthy men residing in Chongqing area of southwest China in 2007. The semen parameters were measured and compared with the World Health Organization (WHO) criteria. A linear regression model was used to examine the determinants of semen quality.

RESULTS: The medians were 2.3 ml for semen volume, 77.8×10^6 per ml for semen concentration, 167.7×10^6 for total sperm count, 33% for sperm rapid progressive motility, 52.6% for sperm progressive motility and 70.9% for total motility. According to WHO criteria, 61.1% of healthy males had at least one semen parameter below normal threshold values. Season and abstinence duration were found to be significantly associated with semen quality ($P < 0.001$). Age, smoking, alcohol use and BMI had little or no effect on semen parameters.

CONCLUSIONS: A high proportion of healthy males in Chongqing area of southwest China had abnormal semen parameters values according to WHO criteria. The semen parameters in the study population were markedly different from those reported for the other Chinese, USA and European populations. The differences remain unexplained and may be due to demographic characteristics, lifestyle, environmental factors or genetic variation.

Key words: semen quality / semen parameters / healthy men / Chinese / risk factor

Introduction

Semen quality is one of the most valuable indications of male reproductive health, and semen analysis plays a critical role in andrology. After a controversial report showing a possible decline in human semen quality over the past 50 years (Carlsen *et al.*, 1992), many countries performed retrospective studies, and many investigators reported a significant reduction in semen quality over time (Auger

et al., 1995; Adamopoulos et al., 1996; Irvine et al., 1996; Menchini-Fabris et al., 1996; Younglai et al., 1998), while several others reported no significant change in human semen quality (Bujan et al., 1996; Fisch et al., 1996; Vierula et al., 1996; Rasmussen et al., 1997). The global temporal trends in semen quality are still being debated. The same arguments about semen quality also arose in China. Zhu et al. (2000) reviewed 36 papers published from 1985 to 1997, analyzed the data from 2318 healthy Chinese subjects, and demonstrated that there was no evidence of a decline in sperm concentration during that 13-year period in China. In contrast, Zhang et al. (1999) showed that there was a trend indicating a decline in semen quality during a 16-year period (1981–1996) in China.

One of the reasons that changes in human semen quality are important is that many studies have indicated that environmental pollutants, especially endocrine disrupters, may alter the development of reproductive organs when males are exposed during fetal and/or neonatal development. Maybe such exposure could impair adult semen production (Toppari et al., 1996; Guo et al., 2000; Ayotte et al., 2001; Hsu et al., 2003). Other studies have emphasized the role of other factors, such as cigarette smoking (Vine et al., 1996, 1994) and various lifestyle factors (Tiemessen et al., 1996) on semen quality. However, increased age may also be associated with a decrease in semen quality, complicating the issue.

Over the past decades, most studies on semen quality have relied on samples from infertility clinics and sperm donor banks (Auger et al., 1995; Bujan et al., 1996). Large studies of the general population are rare. In recent years, several authors have reported large differences in mean sperm concentration between different cities and countries (Fisch et al., 1996; Vierula et al., 1996; Jørgensen et al., 2001; Swan et al., 2003). Gao et al. (2007) have analyzed the semen quality of 1191 healthy men from six provinces in China, but this study did not include Chongqing area of southwest China. Chongqing is one of the largest and most populous of the Chinese municipalities, and contains the majority of the reservoir areas of the Three Gorges Dam project (Three Gorges Reservoir Region). Chongqing is also an important part of the economic zone along the Yangtze River. As a result of its unique geographic characteristics, men from Chongqing may have semen characteristics which are distinct from previous studies. In particular, we are interested in the male reproductive health in this region because it may be related to environmental pollution, especially water-based pollution in the reservoir areas.

The main objectives of this study were: to evaluate the semen quality of a large population of healthy men residing in Chongqing, China; to determine what percentage of these males had normal semen parameters according to the World Health Organization (WHO) criteria and to investigate the effect of selected potential risk factors on semen quality.

Materials and Methods

Study design

Chongqing municipality has a registered population of more than 30 million, and is divided into 40 county-level subdivisions, consisting of 19 districts and 21 counties. We selected three districts and three counties which are geographically and demographically representative of the Three Gorges Reservoir Region, including Wushan and Yunyang counties (located downstream along the Yangtze River in the Three Gorges Reservoir Region); Wanzhou district and Zhongxian county (mid-river); Nanan (upstream of the Yangtze River, and a major urban area of Chongqing) and Shapingba district (a major urban area of Chongqing) (Fig. 1).

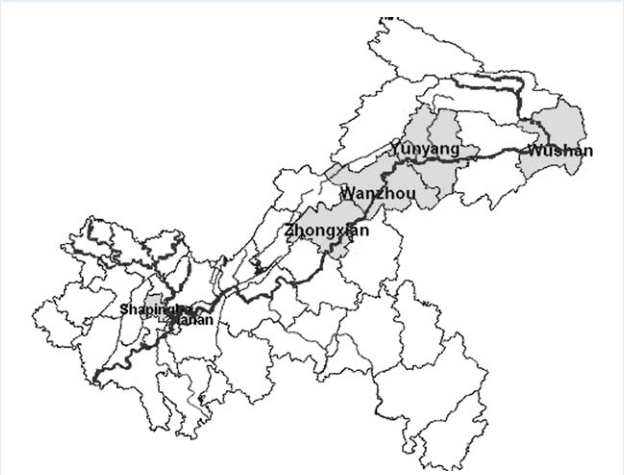

Figure 1 Map of Chongqing area, southwest China.
The colored areas indicate the sampling regions, and the blue lines indicate the Yangzi River and its branches.

This investigation was carried out in 2007. We worked with the Chongqing Family Planning Commission, Chongqing Institute of Science and Technology for Population and Family Planning, and six local Family Planning Institutions to recruit volunteers. A recruitment campaign to enroll participants was organized by the local Family Planning Network. Administration of the questionnaire, and physical examination, semen collection and analysis were carried out at each local Reproductive Health Center. The participants were informed of the purpose of the study, and possible benefits and risks of participating in the study. All participants were asked to sign an informed consent form if they agreed to take part in this study. The project proposal was approved by the Institutional Review Board of Preventive Medicine College, Third Military Medical University.

Study subjects

The subject inclusion criteria were as follows: the men had to be 20–40 years old at the time of inclusion; all men were permanent residents of the participating areas who had not left the area for more than 3 months in past 3 years; the ethnic origin was Chinese Han. The exclusion criteria included: any one of the following reproductive or urological diseases diagnosed by a urologist: hydrocele of the tunica vaginalis, hematocele, hernia, torsion of the spermatic cord, torsion of the testicular appendage, varicocele II or more severe seminal vesiculitis, sexually transmitted disease, gangrene on the skin of the scrotum, cryptorchidism, small testis (<12 ml, testicular volumes were determined by use of a Prader orchidometer), congenital absence of the vas deferens and tuberculosis of epididymis; other known reproductive disorders or an identifiable history of infertility, vasoligation (surgical ligation of the vas deferens as a means of sterilization) or chronic disease; reported duration of abstinence of <2 days or >7 days.

A total of 1976 volunteers were recruited and screened for entry into the study. Of these, 630 were excluded from the analysis for the following reasons: reproductive disorders or other chronic diseases ($n = 80$), missing or unknown duration of abstinence or reported duration of

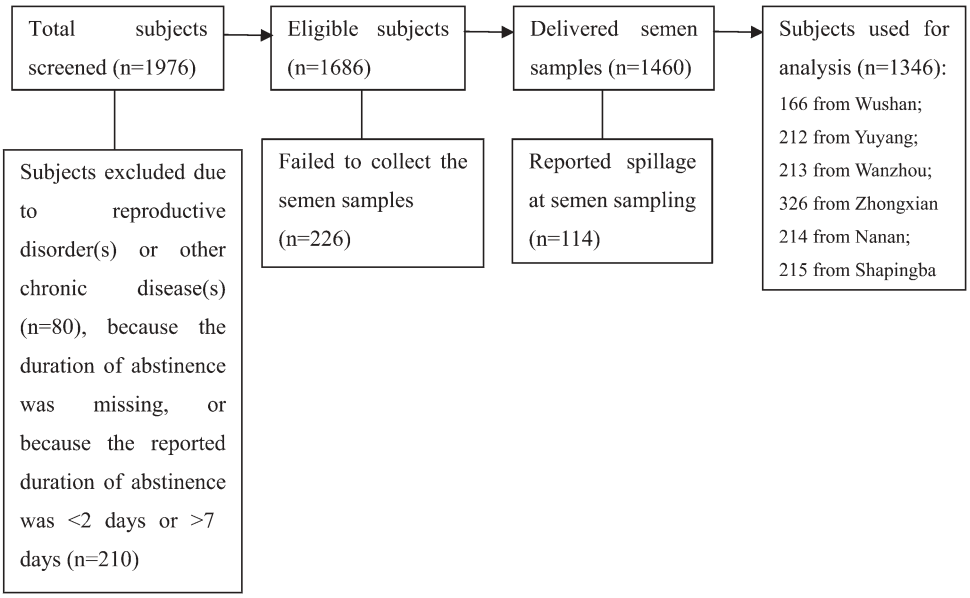

Figure 2 Derivation of the study population.

abstinence of <2 days or >7 days ($n = 210$), failing to collect the semen samples ($n = 226$) and spillage of the sample semen ($n = 114$). Finally, a total of 1346 healthy volunteers were eligible and completed all the steps of the study (Fig. 2).

Questionnaires

The questionnaire included detailed information on demography, education, lifestyle, living conditions, occupational exposure, sexual behavior, reproductive history, the consumption of tobacco, alcohol and drugs, and previous or current diseases. The questionnaire used in this study was standardized for all Reproductive Health Centers.

Physical examination

The physical examinations of all subjects were performed by the same two experienced urologists for all six centers. The results of examinations were recorded in a standard form. Secondary sexual characteristics and the possible presence of a varicocele, a hydrocele, the location of the testis in the scrotum, and the consistency of the testis and epididymis were examined to exclude the subjects with reproductive or urological diseases. Weight (kg) and height (cm) were measured in only one corrected instrument in each center.

Semen collection and analysis

We requested sampling after 2–7 days of abstinence. The exact duration (in days) of abstinence was documented for each patient. The participants collected the ejaculates by masturbation at the local Reproductive Health Center into a sterile, wide-mouth plastic container and immediately delivered the sample to a laboratory in the same building. The semen samples were marked with an anonymous serial number and were then incubated in a water-bath at 37°C until analysis. All samples were analyzed within 60 min of collection.

The semen analyses were performed according to the recommendations of the WHO criteria (World Health Organization, 1999). Semen parameters that were assessed included appearance, viscosity, liquefaction time, pH value, semen volume, sperm concentration, total sperm count and sperm motility. Analysis was started as soon as the ejaculates had liquefied. The volume was measured by aspiration into a 10 ml pipette providing 0.1 ml accuracy. The pH was measured with a pH tape (pH 6.5–10.0) and recorded after 20 s. 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 ×20. The microscope field was scanned systematically and the sperm were classified as either motile (WHO motility classes A, B or C) or immotile (WHO motility class D). For the assessment of sperm concentration, each semen sample was thoroughly mixed. An aliquot of the sample was put into a diluent solution and again thoroughly mixed. The sperm concentration was assessed using a Micro-cell as a counting chamber, and six different areas were counted at a total microscope magnification of ×400. Only sperm with tails were counted.

In order to reduce the variation of assessment of sperm characteristics, all the analyses of semen quality were performed by two well-trained technicians for all six centers using the same apparatus, with one technician evaluating appearance, viscosity, liquefaction time, pH value and semen volume, and the other measuring sperm concentration, motility and morphology. The two technicians participated in the continuous quality control system under the supervision of the Chongqing Science and Technology Commission.

Statistical analysis

Because semen parameters follow markedly skewed (non-normal) distributions, the percentiles, medians and means were calculated on seven semen parameters. Percentages coincident with the criteria of WHO (1999) were also calculated. The data were also summarized using median, 25th and 75th percentiles, and were stratified by age, season of sampling and duration of abstinence. Kruskal–Wallis analysis of variance, a nonparametric test, was used to compare medians between groups.

We then used a linear regression model to examine the independent effects of risk factors on semen parameters. All semen parameters were

log-transformed (base 10) to improve the normality as dependent variables in the linear models. Finally, we back-transformed the regression coefficients for logarithmically transformed variables for ease of interpretation. A full model that included all possible risk factors to be examined in the final regression was used. Selection of risk factors for the final model was based on their importance in the literature and biological plausibility. The possible risk factors, as independent variables, were re-evaluated with dummy variables representing different levels. The independent variables, entered into the regression model, included: age; season (June as summer, September and October as autumn, November and December as winter); duration of abstinence; tobacco (number of cigarettes smoked per day) and alcohol consumption (standard drinks/month, one standard drink equals 10 g of pure alcohol) and BMI (categorized as 18.5–24.9; <18.5 or ≥25 kg/m^2 according to WHO 1997 criteria). The statistical analyses were performed using the Statistical Package for the Social Sciences version 13.0 (SPSS, Chicago, IL, USA). The tests were two-sided, and the level of significance was established at 0.05.

Results

Subject characteristics

The general characteristics of the 1346 eligible subjects are summarized in Table I. Some of the subjects did not complete all of the questions in the questionnaire. The majority of the subjects (71.8%) were 30–40 years old. Nearly half of the subjects had a high school education (46.6%), and had a household income of >3000 RMB (Renminbi, currency of the People's Republic of China) per year (51.2%). More than half of the total subjects used tobacco (61.1%) and alcohol (63.4%). The mean duration of abstinence was 4.5 days. The time from specimen collection to the start of semen analysis averaged 34 min (range: 3–60 min).

Table I Characteristics of participants

Characteristics	*n* (%)
Age (years), *n* = 1345	
20–24	150 (11.2)
25–29	230 (17.1)
30–34	379 (28.2)
35–40	586 (43.6)
Education, *n* = 1335	
Primary school and below	165 (12.4)
Junior school	548 (41.0)
High school	326 (24.4)
College and higher	296 (22.2)
Family income (RMB/year), *n* = 1316	
<3000	642 (48.8)
3000–	378 (28.7)
8000–	193 (14.7)
13 000–	103 (7.8)
Tobacco use (cigarettes/day), *n* = 1308	
No smoking	509 (38.9)
<10	347 (26.5)
≥10	452 (34.6)
Alcohol use (standard drinks/month), *n* = 1346	
No drinks	493 (36.6)
≤120	749 (55.6)
>120	104 (7.8)
Season, *n* = 1346	
Summer (June)	166 (12.3)
Autumn (September, October)	427 (31.7)
Winter (November, December)	753 (55.9)
BMI, *n* = 1338	22.4 (2.9)[a]
Duration of abstinence (days), *n* = 1346	4.5 (1.8)[a]
Time from semen collection to start of analysis (minutes), *n* = 1344	34 (27)[a]

[a]Mean (SD). RMB, Renminbi, currency of the People's Republic of China.

Semen parameters

Table II shows the semen characteristics of the 1346 subjects. The sperm concentration and total count were within high-normal values (94.9 and 91.6%, respectively) according to the WHO criteria. However, a large proportion of the study subjects had sperm progressive motility values below the lower threshold of the WHO criteria. Of the 1346 semen samples evaluated, only 38.9% had all normal semen parameters according to WHO criteria, and 61.1% had at least one of the semen parameters (semen volume, sperm concentration, count, rapid progressive motility and progressive motility) below normal threshold values.

Risk factors for decreased semen quality

The semen samples were grouped separately according to different ages, seasons and duration of abstinence (Table III). The different semen parameters were examined and compared in relation to these variables. Except for pH values ($P < 0.001$), other semen parameters were not significantly different among the different age groups, but some aspects of the semen quality appeared better between 25 and 29 years old. Season significantly affected all of the semen parameters ($P < 0.001$) except for semen volume and sperm rapid progressive motility, and the semen quality in the winter appeared better than in the other two seasons. Semen volume, sperm concentration and total sperm count increased significantly with the duration of abstinence ($P < 0.01$). Sperm rapid progressive motility, progressive motility and total motility reached peak values between 4 and 5 days of abstinence, but the differences were not statistically significant.

Table IV shows adjusted regression coefficients and *P*-values for all possible risk factors in relation to semen parameters. The pH value, sperm concentration and sperm count were significantly associated with the season. The duration of abstinence was related to the pH value, semen volume, sperm concentration and count. Age, smoking, alcohol use and BMI had little or no effect on the semen parameters.

Discussion

In this study, we analyzed the quality of semen from 1346 healthy males (20–40 years old) from Chongqing, China. Our current findings were not in complete agreement with semen parameters observed during several other large studies (number of subjects >500) in

Table II Summary of semen parameters

Semen parameters	*n*	Mean (SD)	Median	Percentiles				Percentage of normal semen parameters according to the WHO criteria[a] (%)
				5	25	75	95	
pH value	1346	7.3 (0.3)	7.2	6.8	7.2	7.5	7.8	77.0
Semen volume (ml)	1341	2.5 (1.3)	2.3	0.8	1.6	3.1	5.0	69.2
Sperm concentration (10^6/ml)	1346	84.8 (59.6)	77.8	19.6	51.0	109.9	168.1	94.9
Total sperm count (10^6)	1346	203.2 (148.3)	167.7	28.0	97.6	270.8	484.4	91.6
Sperm rapid progressive motility (A%)	1346	34.0 (15.6)	33.0	8.3	23.6	44.8	60.0	71.5
Sperm progressive motility [(A+B)%]	1346	51.5 (17.3)	52.6	20.2	40.4	63.5	77.8	57.2
Total motility [(A+B+C)%]	1346	67.3 (20.5)	70.9	27.0	55.0	83.2	94.5	NA

[a]Abnormal values of semen parameters were defined by the World Health Organization (WHO, 1999) standards: pH value <7.2, semen volume <2 ml, sperm concentration $<20 \times 10^6$/ml, sperm total count $<40 \times 10^6$, rapid progressive motility $<25\%$ and sperm progression motility $<50\%$. NA, not available.

Chinese men (Junqing *et al*., 2002; Liu *et al*., 2004; Gao *et al*., 2007; Yan *et al*., 2007). As shown in Table V, the mean and median values of semen volume in our study (2.5 and 2.3 ml, respectively) were in agreement with those of others (2.6 and 2.3–2.8 ml, respectively), but the mean sperm concentration and total sperm count in our study (84.8×10^6/ml and 203.2×10^6, respectively) were markedly higher than those in other studies, ranging from 55.9 to 64.5×10^6/ml, and from 133.6 to 164.2×10^6, respectively. For the mean of total motility, our result (67.3%) was similar to other reports of studies done in Chinese men (70.6–77.2%). In our study, only 38.9% had all normal semen parameters according to WHO criteria. Liu *et al*. (2004) found that 48% sperm donors reached all the WHO reference values of semen parameters, which was higher than that found by Junqing *et al*. (42.3%) and by Gao *et al*. (29.2%). The fact that many studies have shown that a high proportion of populations have abnormal semen parameters has been paid more attention and there has been an increasing opinion that the WHO reference ranges should be reconsidered (Van der Merve *et al*., 2005).

Compared with the values of semen parameters reported in studies of American (USA) and European (France, Denmark, Finland, Estonia and Norway) men (Auger and Jouannet, 1997; Jørgensen *et al*., 2002; Swan *et al*., 2003; Jensen *et al*., 2004) (Table V), the mean semen volume in Chinese men was lower by 0.6–1.4 ml. The mean sperm concentration (84.79×10^6/ml) in our study was higher than was observed in young American and Nordic-Baltic men (range: 54.3–75.5×10^6/ml), but lower than that of French men (95×10^6/ml). The mean total sperm count (203.2×10^6) in our study was higher than was recorded for men in the USA (range: 113.0–149.7×10^6), but was markedly lower than that of French men (337×10^6), and was within the range (from 173 to 235×10^6) of Nordic-Baltic men. For the mean total motility, our study indicated that motility in Chinese men (67.3%) was similar to that of European men (range: 64–73%), but was higher than that in the USA (range: 48.2–56.4%).

The causes of the differences in semen quality among these studies remain under speculation. These variations could be attributable to differences in many factors, such as demographic characteristics, region, lifestyle factors, environmental factors and the methodology used for semen analysis. For example, semen analysis is a rather subjective technique, and is associated with inter-laboratory variation (Jørgensen *et al*., 1997; Swan *et al*., 2003), making it difficult to compare assessments performed by different laboratories. We also cannot exclude geographic variations in semen quality, since several studies have shown apparent geographic variations after adjusting for possible confounders (Fisch *et al*., 1996; Vierula *et al*., 1996; Jørgensen *et al*., 2001; Swan *et al*., 2003). In our study, we also found that there were regional differences in semen quality after adjustment for potential confounders (data not shown), which were consistent with the findings by Junqing *et al*. (2002) and Gao *et al*. (2007). We speculate that these regional variations result from different interactions among lifestyle, other environmental factors and genetic variations, or a combination of these factors. Furthermore, we are also interested in the association of semen quality with environmental pollution, especially water-based pollution in the reservoir areas. We are measuring the exposure levels of priority persistent organic pollutants (POPs) in order to evaluate whether associations between POPs and semen quality in humans are found in this region and thereby evaluate the effects on semen quality.

Our study found that age had no significant effect on semen parameters after adjustment for other potential confounders. However, the correlation between age and semen quality was difficult to assess in our study because the age range (20–40 years old) of participants were restricted. A meta-analysis (Kidd *et al*., 2001) and a recent study (Levitas *et al*., 2007) suggest that increased age is associated with a decrease not only in semen volume, but also in the percentage of normal sperm and sperm motility. Other studies (Eskenazi *et al*., 2003; Carlsen *et al*., 2005) showed that there is no correlation between sperm concentration and male age. The discrepancies among these studies may be due to the different ages of men examined, or due to other confounding factors.

Seasonal variations in semen parameters have been reported in both fertile and infertile men (Levine *et al*., 1988; Saint Pol *et al*., 1989; Centola and Eberly, 1999; Chen *et al*., 2003). Saint Pol *et al*. (1989) found a significant seasonal variation in sperm count, with the highest sperm counts observed in late winter and early spring and the lowest in late summer. In age-adjusted analyses, Centola and Eberly (1999) found significant seasonal variation in the percentage of rapid motile sperm and progressive straight-line velocity, as well as in the percentage of tail defects, immature sperm and

Table III Summary of semen parameters according to age, season and duration of abstinence

Variable	Semen parameters [median (25–75)]						
	pH value	Semen volume (ml)	Sperm concentration (10^6/ml)	Total sperm count (10^6)	Sperm rapid progressive motility (A%)	Sperm progressive motility [(A+B)%]	Total motility [(A+B+C)%]
Age (years)							
20–24	7.2 (7.0–7.4)	2.2 (1.6–3.0)	74.6 (51.5–106.6)	158.4 (98.3–260.8)	36.5 (24.3–44.0)	52.5 (38.3–60.7)	70.9 (54.3–81.1)
25–29	7.2 (7.2–7.5)	2.3 (1.5–3.1)	81.4 (53.5–113.4)	172.3 (103.1–283.2)	34.7 (24.1–46.2)	55.3 (43.8–64.8)	74.5 (58.1–84.0)
30–34	7.2 (7.2–7.5)	2.3 (1.6–3.1)	77.6 (49.1–109.9)	164.2 (94.4–271.3)	32.8 (23.9–45.3)	52.0 (40.2–63.8)	69.6 (52.8–82.7)
35–40	7.2 (7.2–7.5)	2.3 (1.6–3.1)	77.6 (50.9–110.1)	171.0 (97.6–266.5)	32.0 (22.6–44.1)	52.5 (39.2–63.1)	70.0 (54.0–83.2)
P–value[a]	<0.001	0.86	0.79	0.69	0.16	0.29	0.17
Season							
Summer	7.4 (7.2–7.6)	2.5 (1.7–3.2)	52.1 (34.3–79.5)	128.0 (67.5–211.9)	32.2 (23.7–45.5)	49.7 (36.9–62.1)	65.9 (48.3–82.6)
Autumn	7.2 (7.1–7.2)	2.5 (1.7–3.1)	75.6 (50.3–109.2)	160.3 (101.4–269.7)	32.0 (22.5–42.7)	48.8 (36.8–60.5)	65.4 (51.7–80.3)
Winter	7.4 (7.2–7.5)	2.2 (1.5–3.0)	84.2 (57.1–113.8)	184.2 (103.5–279.7)	33.5 (24.5–45.9)	55.3 (44.2–65.0)	73.4 (58.9–84.6)
P–value[a]	<0.001	0.31	<0.001	<0.001	0.08	<0.001	<0.001
Duration of abstinence (days)							
2–3	7.2 (7.2–7.5)	2.1 (1.5–3.0)	72.2 (49.2–102.6)	144.3 (87.4–232.9)	33.3 (24.2–44.0)	52.0 (40.5–62.7)	69.8 (55.3–81.3)
4–5	7.2 (7.1–7.5)	2.5 (1.8–3.2)	79.9 (48.8–111.9)	185.3 (103.3–288.7)	33.6 (23.8–45.5)	53.9 (41.2–64.6)	72.0 (54.0–84.8)
6–7	7.2 (7.2–7.4)	2.5 (1.9–3.2)	82.3 (55.8–116.6)	200.5 (113.2–310.8)	31.8 (22.5–44.8)	51.6 (38.7–63.3)	70.1 (55.1–83.3)
P–value[a]	0.014	<0.001	0.002	<0.001	0.51	0.34	0.22

[a]Kruskal–Wallis analysis of variance was used to compare the median between groups.

Table IV Effects of potential risk factors on semen parameters

Variable	pH value	Semen volume (ml)	Sperm concentration (10^6/ml)	Total sperm count (10^6)	Sperm rapid progressive motility (A%)	Sperm progressive motility [(A+B)%]	Total motility [(A+B+C)%]
Age (years)							
20–24	Ref	Ref	Ref	Ref	Ref	Ref	Ref
25–29	1.01 (0.13)	1.09 (0.15)	1.00 (0.95)	1.07 (0.46)	1.02 (0.72)	1.05 (0.33)	1.08 (0.12)
30–34	1.00 (0.22)	1.08 (0.17)	1.02 (0.75)	1.07 (0.46)	1.00 (0.94)	1.03 (0.50)	1.04 (0.43)
35–40	1.00 (0.82)	1.08 (0.18)	0.99 (0.86)	1.05 (0.59)	0.93 (0.23)	0.98 (0.70)	1.00 (0.92)
Season							
Summer	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Autumn	0.97 (<0.001)	0.99 (0.82)	1.42 (<0.001)	1.36 (<0.001)	0.93 (0.25)	0.99 (0.74)	1.00 (0.98)
Winter	1.00 (0.39)	0.93(0.13)	1.47 (<0.001)	1.30 (<0.001)	0.98 (0.76)	1.07 (0.10)	1.05 (0.21)
Duration of abstinence (days)							
2–3	Ref	Ref	Ref	Ref	Ref	Ref	Ref
4–5	0.99 (0.007)	1.17 (0.04)	1.09 (0.04)	1.27 (<0.001)	1.01 (0.82)	1.02 (0.48)	1.02 (0.42)
6–7	0.99 (0.004)	1.18 (0.04)	1.17 (<0.001)	1.39 (<0.001)	0.98 (0.64)	1.01 (0.87)	1.02 (0.57)
Smoking (cigarettes/day)							
No smoking	Ref	Ref	Ref	Ref	Ref	Ref	Ref
<10	1.00 (0.08)	1.01 (0.72)	0.97 (0.54)	0.97 (0.64)	0.95 (0.30)	1.03 (0.33)	1.02 (0.61)
≥10	1.01 (0.05)	0.97 (0.40)	1.03 (0.53)	0.97 (0.65)	1.03 (0.53)	1.06 (0.05)	1.06 (0.08)
Alcohol use (standard drinks/month)							
No drink	Ref	Ref	Ref	Ref	Ref	Ref	Ref
≤120	1.00 (0.51)	1.04 (0.23)	1.03 (0.49)	1.04 (0.46)	1.01 (0.72)	1.00 (0.94)	0.99 (0.76)
>120	1.00 (0.85)	1.02 (0.72)	0.99 (0.90)	0.95 (0.57)	0.92 (0.21)	0.92 (0.11)	0.91 (0.08)
BMI (kg/m^2)							
≥18.5 and <25	Ref	Ref	Ref	Ref	Ref	Ref	Ref
<18.5	1.00 (0.94)	1.00 (0.98)	0.92 (0.29)	0.90 (0.28)	1.00 (0.96)	0.99 (0.78)	0.96 (0.49)
≥25	1.00 (0.97)	0.94 (0.13)	0.96 (0.37)	0.92 (0.22)	1.05 (0.28)	1.05 (0.14)	1.00 (0.96)

Values shown are the coefficients and *P*-values. The coefficients were back-transformed to display the relative differences from the chosen reference groups (y|x=other group)/(y|x=reference group). Ref, reference.

tapered sperm. Chen *et al*. (2003) also reported seasonal variations in sperm concentration. The seasons of our study included several months representative of summer, autumn and winter. In agreement with previous studies, our results showed that semen volume, sperm concentration and sperm count were significantly associated with season, with better semen quality in the winter than in the other two seasons. There are several possible explanations for seasonal variation in semen parameters. Some investigators consider that semen quality is subject to seasonal changes because of temperature changes (Politoff *et al*., 1989; Chia *et al*., 2001) or the length of daylight (Snyder *et al*., 1990). A recent report (Carlsen *et al*., 2004) suggested that ejaculatory frequency may be the key reason for seasonal variation in sperm concentration. We are currently investigating the effect of season on sperm morphology and other biologic markers in our samples.

It has been well-demonstrated that the duration of abstinence can influence semen quality. Gao *et al*. (2007) found that semen volume and sperm concentration were increased in the subjects after 4–7 days of abstinence, which was consistent with our results. Levitas *et al*. (2005) evaluated the relationship between the duration of abstinence (1–14 days) and various characteristics of normal and subnormal semen, and found that mean values of semen volume, sperm concentration, total sperm count, percentage of motile sperm and total motile sperm count per ejaculation were related to the duration of abstinence in each group, and that an increase in the duration of abstinence from 3 to 6 days is positively and significantly related to sperm concentration. Our finding that total motility was not significantly related to the duration of abstinence may be due to the strict limitation of 3–7 days of abstinence in our study.

In our study, tobacco and alcohol consumption did not appear to be associated with semen parameters. This is consistent with several other studies (Dikshit *et al*., 1987; Martini *et al*., 2004; Gao *et al*., 2007). However, many studies showed associations between male smoking and sperm concentration and motility (Vine *et al*., 1996, 1994; Said *et al*., 2005; Ramlau-Hansen *et al*., 2007). Said *et al*. (2005) reported a negative effect of tobacco on semen quality, although in their study this decrease was related to tobacco chewing and not to cigarette smoking. More recently, Ramlau-Hansen *et al*.

Table V Summary of semen parameters of Chinese, European and American men

Study (year published)	Period of study	Region	Number of subjects (age, years)	Selection of subjects	Semen parameters							
					Semen volume (ml)		Sperm concentration (10^6/ml)		Total sperm count (10^6)		Total motility (A+B+C)%]	
					Mean	Median	Mean	Median	Mean	Median	Mean	Median
Studies in China												
Our study	2007	Chongqing	1346 (20–40)	Healthy general population	2.5	2.3	84.8	77.8	203.2	167.7	67.3	70.9
Gao *et al.* (2007)	2000–2002	Hebei, Henan, Shanxi, Zhejiang, Qindao, Guizhou	1191 (20–60)	Healthy general population	–	2.3	–	65	–	154	–	67
Yan *et al.* (2007)	2005	–	1054 (18–35)	Healthy army men	2.6	–	55.9	–	133.6	–	70.6	–
Liu *et al.* (2004)	2004	Guangdong	512 (22–45)	Sperm donors	–	2.8	–	73.9	–	146. 4	–	–
Junqing *et al.* (2002)	1998–2002	Shanghai, Henan, Zhejiang, Shanxi, Shandong, Hebei, Guizhou	562 (22–30)	Healthy volunteers	2.6	–	64.5	–	164.2	–	77.2	–
Studies in Europe												
Jensen *et al.* (2004)	1996–1998	Denmark	1558 (mean: 19)	Healthy males from the compulsory military medical examination	3.2	–	–	44	–	128	65.2	–
Jørgensen *et al.* (2002)	1997–1999	Denmark	300 (18–20)	Healthy males from the compulsory military medical examination	3.3	3.0	57	44	173	130	65	68
		Finland	324 (18–20)		3.3	3.0	72	61	221	194	64	66
		Estonia	104 (18–20)		3.2	3.1	72	62	235	180	73	75
		Norway	240 (18–20)		3.1	2.9	69	53	205	158	64	66
Auger and Jouannet (1997)	1973–1993	France	4710 (mean: 35)	Healthy volunteers	3.7	3.4	95	80	337	264	64	65
Studies in USA												
Swan *et al.* (2003)	1999–2001	Missouri	176 (mean: 30.7)	Fertile Males	3.9	–	54.3	–	113.0	–	48.2	–
		California	124 (mean: 29.8)		3.6	–	69.0	–	137.5	–	54.5	–
		Minnesota	155 (mean: 32.2)		3.9	–	74.6	–	152.9	–	52.1	–
		New York	38 (mean: 36.1)		3.3	–	75.5	–	149.7	–	56.4	–

–, not available.

(2007) reported that smoking was a risk factor for decreased semen quality, and observed a statistically significant dose–response relationship between current cigarette smoking and several semen characteristics. One earlier study (Pajarinen *et al*., 1996) indicated that long-term average daily consumption of <40 g of alcohol seemed not to be associated with disorders of spermatogenesis, whereas high alcohol consumption might be associated with serious disorders of spermatogenesis. Results reported by a recent study (Muthusami and Chinnaswamy, 2005) demonstrated that chronic alcohol consumption has a detrimental effect on male reproductive hormones and on semen quality. There is no clear explanation for these differences. One possibility is that the levels of tobacco and pattern of alcohol consumption were different among these studies. Further investigations are needed to determine whether tobacco and alcohol consumption play a role in decreasing semen quality.

Several studies have demonstrated that BMI could be associated with male fertility. Jensen *et al*. (2004) found a significant association between sperm count and BMI, as overweight as well as overly slim men had lower sperm concentrations and also lower total sperm counts compared with men with ideal body weights (BMI between 20 and 25 kg/m^2). Results reported by Kort *et al*. (2006) revealed a significant and negative relationship between BMI and the total number of normal-motile sperm cells, and men presenting with a BMI >25 kg/m^2 have fewer chromatin-intact normal-motile sperm cells per ejaculate. In our study, BMI did not appear to have an effect on semen quality, which is consistent with the results on the Chinese population reported by Gao *et al*. (2007). The different obesity prevalence rates among races in different countries may cause the discrepancies among these studies, since obesity prevalence rates are known to be very high in industrialized areas. Further research is needed on exactly how obesity affects semen production. A recent study (Aggerholm *et al*., 2008) found that overweight and obese men have a markedly changed sex hormone profile in serum. Many factors, such as different hormone levels in obese men, overheating of the testicles caused by excessive fat in the area or the lifestyle and diet that leads to obesity could affect semen quality.

The most important strength of our study is the sample size; the inclusion of 1346 healthy men made this study one of the largest ever in a Chinese population. Furthermore, our study was based on a community population, unlike most of the previous studies, which were based on clinic or hospital populations, or sperm donor banks. Several studies (Larsen *et al*., 1998; Lalos *et al*., 2003; Muller *et al*., 2004) have reported that there are major differences in demographic characteristics between semen donors and the general population.

We realize that our study was not without limitations. One apparent limitation of the study was the fact that only one semen sample was evaluated for each subject. However, since the number of subjects evaluated was relatively high, this would tend to minimize the potential effect of sample variability of semen quality. The other main limitation is that the subjects were not randomly selected, but volunteers. Therefore, demographic characteristics, including the drinking and smoking rates of our study population, may be different from the general population, as volunteers may tend to be those who are concerned about their fertility. Because the subjects were recruited by local Family Planning Institutions, we cannot estimate the participation rate. Our study did not include a questionnaire for the men refusing to participate, so the reason(s) for their refusal are not known.

The present study was one of largest studies on semen quality in a Chinese population, and focused on representative regions of the Three Gorges Reservoir Region in Chongqing in southwest China, which has not been evaluated before. This investigation showed that a high proportion of Chinese healthy males (61.1%) had abnormal semen parameters values, according to WHO criteria. For the mean of semen volume and sperm total motility, our results were similar to other reports of studies in Chinese men, but the mean sperm concentration and total sperm count in our study were markedly higher than in other studies in Chinese men. Compared with the values reported by studies carried out in the USA and Europe, the mean semen volume in Chinese men was markedly lower. We did not find any significant association of the poor semen quality with smoking, drinking alcohol or BMI, but did note that season and the duration of abstinence may be important factors that influence semen quality. This study provides important basic data on male reproductive health in southwest China, and will provide a large population data set for comparison of semen quality in different regions of China or in different ethnic populations.

Authors' contribution

J.C. conceived and designed this study, and helped with interpretation of results and preparation of the manuscript. Y.L. drafted the manuscript, collected and analyzed the data. H.L. contributed to analysis of data and revising the article. M.C. and H. B. analyzed the semen quality and interpreted the data. C.L. and H.Y. performed physical examinations and interpreted the data. M.M., L.L., N.Z., X.H., L.H., C.Z., Z.R. and Y.X. contributed to acquisition of data and revising the article. Z. C., L.A., Z.Z. and H.X. helped to design this study and supplied important comments. All authors gave the final approval of the version to be published.

Acknowledgements

We are grateful to the Chongqing Family Planning Commission, and six local Family Planning Institutions and Reproductive Health Centers (Wushan, Yunyang, Wanzhou, Zhongxian, Nanan and Shapingba) for coordinating the recruitment of participants. We acknowledge the contributions of all staff members of the Department of Epidemiology and the Department of Hygienic Toxicology, Third Military Medical University. We thank Dr Botao Guo (Department of Health Statistics, Third Military Medical University) for advice on statistical analyses and Dr R. Zhang (Department of Pharmacology and Toxicology, University of Alabama, Birmingham, USA) for helpful comments. We also thank all the volunteers for participating in the study.

Funding

This research was supported by the Key Project of National Natural Science Foundation of China (No. 30630056) and the Program of S&T (Chongqing) Project (No. CSTC 2006AA703).

References

Adamopoulos DA, Pappa A, Nicopoulou S, Andreou E, Karamertzanis M, Michopoulos J, Deligianni V, Simou M. Seminal volume and total sperm number trends in men attending subfertility clinics in the greater Athens area during the period 1977–1993. *Hum Reprod* 1996;**11**: 1936–1941.

Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH, Bonde JP. Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertil Steril* 2008; **90**:619–626.

Auger J, Jouannet P. Evidence for regional differences of semen quality among fertile French men. *Hum Reprod* 1997;**12**:740–745.

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

Ayotte P, Giroux S, Dewailly E, Hernández Avila M, Farias P, Danis R, Villanueva, Díaz C. DDT spraying for malaria control and reproductive function in Mexican men. *Epidemiology* 2001;**12**:366–367.

Bujan L, Mansat A, Pontonnier F, Mieusset R. Time series analysis of sperm concentration in fertile men in Toulouse, France between 1977 and 1992. *BMJ* 1996;**312**:471–472.

Carlsen E, Giwercman A, Keiding N, Skakkebaek N. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; **305**:609–613.

Carlsen E, Petersen J, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. *Fertil Steril* 2004;**82**:358–366.

Carlsen E, Swan SH, Petersen JH, Skakkebaek NE. Longitudinal changes in semen parameters in young Danish men from the Copenhagen area. *Hum Reprod* 2005;**20**:942–949.

Centola GM, Eberly S. Seasonal variations and age-related changes in human sperm count, motility, motion parameters, morphology, and white blood cell concentration. *Fertil Steril* 1999;**72**:803–808.

Chen Z, Toth T, Godfrey-Bailey L, Mercedat N, Schiff I, Hauser R. Seasonal variation and age-related changes in human semen parameters. *J Androl* 2003;**24**:226–231.

Chia SE, Lim ST, Ho LM, Tay SK. Monthly variation in human semen quality in male partners of infertile women in the tropics. *Hum Reprod* 2001;**16**:277–281.

Dikshit RK, Bush JG, Mansuri SM. Effect of tobacco consumption on semen quality of a population of hypofertile males. *Fertil Steril* 1987; **48**:334–336.

Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D. The association of age and semen quality in healthy men. *Hum Reprod* 2003;**18**:447–454.

Fisch H, Feldshuh J, Goluboff ET, Broder SJ, Olson JH, Barad DH. Semen analyses in 1,283 men from the United States over a 25-year period: no decline in quality. *Fertil Steril* 1996;**65**:1009–1014.

Gao J, Gao ES, Yang Q, Walker M, Wu JQ, Zhou WJ, Wen SW. Semen quality in a residential, geographic and age representative sample of healthy Chinese men. *Hum Reprod* 2007;**22**:477–484.

Guo YL, Hsu PC, Hsu CC, Lambert GH. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. *Lancet* 2000;**356**:1240–1241.

Hsu PC, Huang W, Yao WJ, Wu MH, Guo YL, Lambert GH. Sperm changes in men exposed to polychlorinated biphenyls and dibenzofurans. *JAMA* 2003;**289**:2943–2944.

Irvine S, Cawood E, Richardson D, MacDonald E, Aitken J. Evidence of deteriorating semen quality in the United Kingdom: birth cohort study in 577 men in Scotland over 11 years. *BMJ* 1996;**312**:467–471.

Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril* 2004;**82**:863–870.

Jørgensen N, Auger J, Giwercman A, Irvine DS, Jensen TK, Jouannet P, Keiding N, Le Bon C, MacDonald E, Pekuri AM *et al.* Semen analysis performed by different laboratory teams: an intervariation study. *Int J Androl* 1997;**20**:201–208.

Jørgensen N, Andersen AG, Eustache F, Irvine D, Suominen JSS, Petersen JH, Andersen AN, Auger J, Cawood EHH, Horte A *et al.* Regional differences in semen quality in Europe. *Hum Reprod* 2001; **16**:1012–1019.

Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, Andersson AM, Haugen TB, Horte A, Jensen TK. 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**:2199–2208.

Junqing W, Qiuying Y, Jianguo T, Wei Y, Liwei B, Yuxian L, Yumei Z, Kangshou Y, Weiqun L, Lu C *et al.* Reference value of semen quality in Chinese young man. *Contraception* 2002;**65**:365–368.

Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril* 2001;**75**: 237–248.

Kort HI, Massey JB, Elsner CW, Mitchell-Leef D, Shapiro DB, Witt MA, Roudebush WE. Impact of body mass index values on sperm quantity and quality. *J Androl* 2006;**27**:450–452.

Lalos A, Daniels K, Gottlieb C, Lalos O. Recruitment and motivation of semen providers in Sweden. *Hum Reprod* 2003;**18**:212–216.

Larsen SB, Abell A, Bonde JP. Selection bias in occupational sperm studies. *Am J Epidemiol* 1998;**147**:681–685.

Levine RJ, Bordson BL, Mathew RM, Brown MH, Stanley JM, Starr TB. Deterioration of semen quality during summer in New Orleans. *Fertil Steril* 1988;**49**:900–907.

Levitas E, Lunenfeld E, Weiss N, Friger M, Har-Vardi I, Koifman A, Potashnik G. Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertil Steril* 2005; **83**:1680–1686.

Levitas E, Lunenfeld E, Weisz N, Friger M, Potashnik G. Relationship between age and semen parameters in men with normal sperm concentration: analysis of 6022 semen samples. *Andrologia* 2007; **39**:45–50.

Liu M, Ma C, Tang L, Wen R, Deng S, Wang Q, Jiang Y, Chen A. Quality analysis of the primary semen samples from 512 donors [in Chinese]. *Zhonghua Nan Ke Xue* 2004;**10**:734–736.

Martini AC, Molina RI, Estofan D Senestrari D, Fiol de Cuneo M, Ruiz RD. Effects of alcohol and cigarette consumption on human seminal quality. *Fertil Steril* 2004;**82**:374–377.

Menchini-Fabris F, Rossi P, Palego P, Simi S, Turchi P. Declining sperm counts in Italy during the past 20 years. *Andrologia* 1996;**28**: 304.

Muller A, De La Rochebrochard E, Labbe-Decleves C, Jouannet P, Bujan L, Mieusset R, Le Lannou D, Guerin JF, Benchaib M, Slama R *et al.* Selection bias in semen studies due to self-selection of volunteers. *Hum Reprod* 2004;**19**:2838–2844.

Muthusami KR, Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. *Fertil Steril* 2005;**84**:919–924.

Pajarinen J, Karhunen PJ, Savolainen V, Lalu K, Penttilä A, Laippala P. Moderate alcohol consumption and disorders of human spermatogenesis. *Alcohol Clin Exp Res* 1996;**20**:332–337.

Politoff L, Birkhauser M, Almendral A, Zorn A. New data confirming a circannual rhythm in spermatogenesis. *Fertil Steril* 1989;**52**: 486–489.

Rasmussen PE, Erb K, Westergaard LG, Laursen SB. No evidence for decreasing semen quality in four birth cohorts of 1,055

Danish men born between 1950 and 1970. *Fertil Steril* 1997; **68**:1059–1064.

Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. *Hum Reprod* 2007;**22**:188–196.

Said TM, Ranga G, Agarwal A. Relationship between semen quality and tobacco chewing in men undergoing infertility evaluation. *Fertil Steril* 2005;**84**:649–653.

Saint Pol P, Beuscart R, Leroy-Martin B, Herman E, Jablonski W. Circannual rhythms of sperm parameters of fertile men. *Fertil Steril* 1989;**51**:1030–1033.

Snyder PJ. Fewer sperm in the summer—it's not the heat, It's. . .. *N Engl J Med* 1990;**323**:54–56.

Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW, and Study For Future Families Research Group. Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect* 2003;**111**:414–420.

Tiemessen CH, Evers JL, Bots RS. Tight-fitting under-wear and sperm quality. *Lancet* 1996;**347**:1844–1845.

Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJ Jr, Jégou B, Jensen TK, Jouannet P, SumpterM J *et al*. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 1996;**104**(Suppl. 4):741–803.

Van der Merve FH, Kruger TF, Oehninger SC, Lombard CJ. The use of semen parameters to identify the subfertile male in the general population. *Gynecol Obstet Invest* 2005;**59**:86–91.

Vierula M, Niemi M, Keiski A, Saaranen M, Saarikoski S, Suominen J. High and unchanged sperm counts of Finnish men. *Int J Androl* 1996; **19**:11–17.

Vine MF. Smoking and male reproduction: a review. *Int J Androl* 1996; **19**:323–337.

Vine MF, Margolin BH, Morrison HI, Hulka BS. Cigarette smoking and sperm density: a meta-analysis. *Fertil Steril* 1994;**61**:35–43.

World Health Organization. WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. *Collection and Examination of Human Semen*. Cambridge: Cambridge University Press, 1999, 4–14.

Yan S, Tang J, Zhang N, Wang X, Lu H, Wang M. Investigation of Semen Quality of 18–35 Years Old Chinese Army Men [in Chinese]. *Zhonghua Nan Ke Xue* 2007;**13**:134–137.

Younglai EV, Collins JA, Foster WG. Canadian semen quality: an analysis of sperm density among eleven academic fertility centers. *Fertil Steril* 1998; **70**:76–80.

Zhang SC, Wang HY, Wang JD. Analysis of change in sperm quality of Chinese fertile men during 1981–1996 [in Chinese]. *Reprod Contracept* 1999;**10**:33–39.

Zhu Xinqiang, Shen Yi, Zhang Xinyang, Chang Yan, Huang Xingshu. Trend analysis of sperm quality during the past 13 years in China [in Chinese]. *J Zhejiang Univ* 2000;**29**:173–176.

Submitted on August 28, 2008; resubmitted on October 7, 2008; accepted on October 13, 2008