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Semen quality analysis of military personnel from six geographical areas of China

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Capsule:

This is the first attempt in China to examine the determinants of semen quality by investigating a large sample of military servicemen from different geographical areas. Lower semen parameters were found and potential influences were putatively identified.

Abstract

Objective: To examine the determinants of semen quality ~~in by investigating~~ a large sample of military ~~personnel servicemen~~ from different geographical areas of China.

Design: Cross-sectional study.

Setting: Six representative geographical regions in China: Beihai, Lhasa, Germu, Xinzhou, Huhehaote and Mohe.

Subjects: 1194 ~~army military personnel aged servicemen from six geographical areas of China, who were~~ 18–35 years of age at the time of their inclusion ~~in the study, sampled and serving in the ordinary land forces from between~~ 2007 ~~and~~ –2009.

Intervention(s): None.

Main Outcome Measure(s): Semen volume (in milliliters), sperm concentration (in millions per milliliter), percentage of motile spermatozoa, total sperm count (in millions), and relative risk of subfertility ~~for military servicemen~~.

Result(s): The median ~~values~~ were 3.0 ml for semen volume, 39.4×10^6 per ml for sperm concentration, 120.1×10^6 for total sperm count, 15.8% for sperm rapid progressive motility, 30.1% for sperm progressive motility and 43.9% for total motility. ~~According to WHO recommendations (1999),~~ 88.3% of the servicemen had at least one semen parameter below normal ~~threshold~~ values ~~according to WHO recommendations (1999), and according to WHO recommendations (2010),~~ 62.5% of the servicemen ~~according to WHO recommendations (2010) had at least one semen parameter below the lower reference limits.~~ Season, average altitude and ~~duration of abstinence all duration~~ were found to be significantly associated with semen quality ($P < 0.001$).

Conclusion(s): The subjects had markedly lower mean sperm concentrations, sperm counts and sperm motility compared to ~~the~~ WHO recommendations. ~~Possible c~~Contributory factors to their reduced sperm quality included diet, lifestyle, climate and altitude.

Key Words: Semen quality; semen parameters; Chinese military [personnel](#)

~~servicemen~~

Based on 61 reports published between 1938 and 1990, ~~in 1992~~ Carlsen et al. (1) conducted a meta-analysis which indicated a significant general decrease in mean sperm concentration from 113 million/ml in 1940 to 66 million/ml in 1990. While some subsequent studies have confirmed ~~this~~ declining trend in sperm concentration others have failed to detect a temporal trend (2–15), with the contradictory results variously attributed to sample selection bias, regional differences and/or experimental error (16–19).

To our knowledge, most studies were based on subjects who were long-term or permanent residents of a region ~~and/or~~ were recruited from the general population (10,19). With globalization of the world economy, population mobility has been increasing rapidly and by 2007 ~~it was estimated that~~ there were estimated to be 120 million internal migrants in China (20). Given these numbers, reproductive function in the male Chinese migrant population has become an important health issue. More generally, changes in patterns of migrant fertility may reveal the influence of environmental factors and lifestyle on semen quality.

~~Military conscription is compulsory for males (correct??) In China all and for military personnel service conscripts~~ are posted to a location at some distance from their home province. Since all servicemen share similar living environments and lifestyles, according to the IUSSP criteria of Multilingual Demographic Dictionary (21) they can be regarded as representative of an example of a migrant population within China. We therefore decided to evaluate the semen quality of a large cross-section of ~~the~~ military personnel serving in the People's Liberation Army (PLA), to determine the what percentage who had normal semen parameters according to the World Health Organization (WHO) recommendations and to investigate the effect of selected potential risk factors on semen quality.

MATERIALS AND METHODS

Study Design

China has a wide variety of geographical environments, including tropical and cold zones, upland plateaus and plains, and coastal and inland regions. We selected six districts where military personnel are posted serve in the PLA aand which are geographically representative of the country's regional characteristics, i.e. Beihai (Guangxi province, coastal tropical zone), Lhasa (Tibet autonomous region, cold plateau), Germu (Qinghai province, cold plateau), Xinzhou (Shanxi province, inland plains), Huhehaote (Inner Mongolia autonomous region, inland plains), and Mohe (Heilongjiang province, cold inland). More detailed The climatic and geographical characteristics of these districts are provided shown in Supplemental Table 1.

Sample collection occurred from September 16-23, 2009 in Beihai, September 3-12, 2007 in Lhasa, January 7-16, 2008 in Mohe, July 18-29, 2008 in Germu, October 9-17, 2009 in Xinzhou, October 18-27, 2009 in Huhehaote, and January 7-16, 2008 in Mohe (why not list chronologically?). Administration of the questionnaire, physical examinations, and semen collection and analysis were undertaken conducted at the appropriate each local military family planning institutions by the same two staff members of of the Airforce General Hospital, PLA??, China. Ethical approval was provided obtained by from the General Airforce General Hospital Ethical Committee, Beijing, China, which supervises all six regional centers. Approval also was obtained from the Ethics Committees of Capital Medical University, Beijing, and the Airforce General Hospital PLA, Beijing, China??. Each participant voluntarily signed an informed consent form after the nature of the proposed project was explained in the local test sites military Family Planning Institutions. The participation rates in the study were relatively high, with little variation between the different regions, i.e. ranging from 80.4% in Beihai to 85.6% in Lhasa.

Subjects

A total of 1438 servicemen were initially recruited and screened for entry into the study, with 244 (16.9%) excluded due to reproductive disorders (213, 14.8%) or incomplete duration of abstinence (31, 2.1%). All 1194 eligible volunteers who completed the study were Han Chinese drawn from 26 different provinces of China, with 616 volunteers from northern China and 578 from southern China. All participants had been stationed for at least one year at their sampling locations, which as previously noted were distant from their birthplace or former living place. A detailed description of the inclusion and exclusion criteria of the study is provided in the Supplemental Materials and Methods.

Questionnaire

The standardized questionnaire used in all centers comprised detailed information on demographic background, lifestyle factors, military service time, occupational exposure (routine duties e.g. drivers, radar serviceman etc),²² sexual history, reproductive history, the consumption of tobacco, alcohol and drugs, and previous or current diseases.

Physical examination

All participants had previously been subject to a general medical examination on entry to military service, and for the study they, ~~Participants in the study~~ were examined by the same specialist urologist at the time of their participation. 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 subjects with reproductive or urological diseases.

Semen analysis

Semen samples were assessed according to the WHO 1999 recommendation (22). After liquefaction and within 1h of ejaculation the samples were analyzed for semen volume and pH, sperm concentration and motility (defined as WHO motility grades A, B, C and D).

Statistics

As semen parameters follow markedly skewed (non-normal) distributions, unadjusted mean and median values, SD and 25th–75th percentiles were calculated for each variable. Percentages coincident with ~~WHO~~the recommendations ~~of WHO~~(1999, 2010) were also calculated and stratified according to military service location. A nonparametric Kruskal–Wallis analysis of variance was used to compare between-group medians. The effects of possible confounders were tested by ~~a~~multiple regression analysis, initially for all six groups combined and then by individual location. ~~The~~ tests were two-sided, and the level of significance was established at 0.05. Further details of the physical examinations, semen analyses and statistical assessments are provided in Supplemental Materials and Methods.

RESULTS

The general biological characteristics of the 1194 subjects are summarized in Table 1. A large majority of subjects (95.9%) were 18–30 years old, ~~and their~~ mean ages ~~of all participants~~ ranged from 24.3 years old (Lhasa) to 26.4 years old (Xinzhou), ~~and their mean period of military service was 5.3 years.~~ 1075 (90.0%) participants reported zero alcohol consumption and few participants (~~2~~2.6%) had an average daily consumption of tobacco exceeding 10 cigarettes. Only 23 (1.9%) participants were married. ~~The mean period of military service was 5.3 years, and T~~the mean duration of any form of sexual abstinence was 4.5 days (~~2-7~~2-7 days).

The time from specimen collection to the start of semen analysis averaged 29 minutes. The semen characteristics of the 1194 subjects are listed in Tables 2 and 3. Participants based in Xinzhou had the highest mean sperm concentration (~~numbers~~ $69.7 \times 10^6/\text{ml}$) and those in Lhasa the lowest (~~numbers~~ $51.5 \times 10^6/\text{ml}$), but the difference was not statistically significant. The semen volume, total sperm count and sperm rapid progressive motility in participants from

Lhasa were lower than those of other centers: (~~Significant???~~Significant, $P < 0.05$). There were few differences in the mean semen characteristics of participants by age, military service, birthplace or average air temperature over last five years. Mean values for sperm concentration and rapid progressive motility did, however, show significant seasonal differences, with the lowest values in autumn. Mean semen volume, sperm concentration and total sperm count were all higher in sites located at lower altitude ($\leq 1000\text{m}$) than higher altitude ($> 1000\text{m}$). The sperm concentration and total sperm count increased significantly with the duration of sexual abstinence, reaching peak values between four and five days.

Table 4 shows adjusted regression coefficients and P -values for possible risk factors in relation to semen parameters. ~~The duration of a~~Sexual abstinence duration was related to ~~the~~ sperm concentration and count (repetition??), while the season significantly affected all ~~of the~~ semen parameters except for semen volume. ~~The s~~Semen volume, sperm concentration, sperm count and sperm rapid progressive motility were significantly associated with average altitude, age, military service time and average air temperature of the last five years, whereas birthplace had no effect on the semen parameters. (Contradiction of previous para?)

DISCUSSION

~~The Our~~ study showed significantly lower proportions of sperm concentrations (74.1%), counts (79.6%) and motility (43.7%) than the WHO reference ranges (1999), and only 11.7% of participants had all normal semen parameters according to these WHO recommendations. More recently WHO (2010 Year???2010) revised the semen reference intervals (????22)—by studying the semen parameter distributions of men whose partners had a time-to-pregnancy (TTP) up to and including 12 months. The new criteria of semen parameters, including such as semen volume, sperm concentration, total sperm number, total motility and progressive motility (22,23) are lower

than ~~those in~~ the previous WHO (1999) recommendations. However, the semen parameters indicated by ~~of~~ our study also are ~~are still~~ lower than the WHO (2010) recommendations, with ~~:-~~ Compared to the 2010 WHO reference intervals, 94.7% of semen volumes, 80.3% of sperm concentrations, 80.0% of total sperm counts, 46.5% of progressive motility, and 54.6% of total motility (reached over) are above the low limits (???). Only 37.5% of the individuals attained normal semen parameters in all tests.

As shown in Table 2, the respective mean and median values of sperm concentration, count and total motility are also markedly lower when compared to earlier reports from subjects recruited ~~drawn~~ from healthy general populations (24-29). In other studies on military personnel, Punab et al. (19) reported that the sperm concentrations of 118 Estonian soldiers (106 million per ml) was higher than that of the Lithuanian and Estonian general populations. [Obviously our data are lower than all these reports]. Our results are in agreement with Yan et al. who investigated ~~reported the semen parameters of~~ 1054 Chinese servicemen from a single army cohort and reported ~~showing that the~~ mean sperm concentration, sperm count and total motility values of were $55.9 \times 10^6/\text{ml}$, 133.6×10^6 and 70.6%, respectively (30), ~~which were in agreement with our investigation~~, but no data on median values were ~~not~~ provided.

The main strengths of the present study are the ~~overall~~ large sample size, high overall participation rate and relative conformity of the sub-populations tested, Multiple regression analysis was used to control for confounders, first for all six groups combined and then by ~~for~~ each individual sub-group. Although some confounding may remain, we feel that it would be unlikely to explain the apparent scale of the lower sperm quality detected. Although ~~s~~The servicemen generally have better overall general health condition than ~~conscripts~~ than their peers drawn from the general population, they also show ~~but~~ relatively lower sperm concentrations, counts and motility.

The present subjects all had served in the army ~~PLA land forces~~ for more than one year and had

~~were~~ not involved in combat missions when the semen samples were collected. None of the participants were exposed to factors such as scrotal heating or other ~~potentially special-adverse harmful~~ physical or chemical environments. As personnel enlisted from their native provinces for service elsewhere in China have to adapt to changes ~~in such as dietfood,~~ living habits, climate and altitude, ~~it seemed possible we speculate~~ that these changes ~~mayeould have~~ contributed to differences in semen quality. [Moreover, the adaption processes could not improve the sperm quality (there were not significant differences of semen characteristics among different service time groups) ~~????~~.] (I agree that we can remove this sentence)!

~~In our study, we found that the subjects from Lhasa have the poorest semen quality, especially in terms of the semen volume, total sperm count and sperm rapid progressive motility (A%).~~ Iwasaki et al. had suggested that anoxia and UV exposure exert negative effects on the sperm function of male adults (31), ~~and in our study the personnel stationed in Lhasa had the poorest semen quality, especially in terms of the semen volume, total sperm count and sperm rapid progressive motility (A%).~~ The regression coefficients for altitude between groups of above and below 1000 meters in relation to the semen volume, sperm concentration, total sperm count and sperm rapid progressive motility (A%) ~~we~~ are all less than 1 ($P_p=0.0001$). The higher the altitude, i.e. the greater the exposure to anoxia and UV, ~~and possibly related to physical performance,~~ the lower the mean (~~median??~~) semen volume, sperm concentration and motility, suggesting that altitude is a risk factor for certain specific semen characteristics, especially semen volume and sperm rapid progressive motility, which is consistent with the ~~altitude-based study report by from~~ Yu et al. (32).

Whether ~~or not there is~~ any correlation exists between semen parameters and age is controversial (33-36), ~~however in .—Oour study found that~~ the age of the subjects, ~~from 18 to 35 years old with and 96.5% between 18-30 years,~~ had no significant effect on semen parameters.

~~Substantial significant~~ differences in the age ranges of subjects in previous investigations may have been ~~at~~ the major cause for conflicting findings.

~~Our results showed that the S~~perm concentrations, sperm counts and motility ~~were~~ all significantly lower in autumn than in summer, with no significant differences between winter and summer. We also found that the semen characteristics had no significant differences between the ~~two~~ groups (~~X and Y~~) Beihai, Lhasa, Germu, Xinzhou, Huhehaote, and Mohe where the average air temperature over the last five years were above and below 0°C (~~???~~ Around 0°C), ~~suggesting either indicating~~ that the average yearly air temperature ~~had~~s little influence on semen quality, or that males could adapt to air temperature changes. Many investigators have attributed seasonal variations in sperm concentration to temperature changes, ~~the~~ daylight length, or differences in ejaculatory frequency (36-39). Our results suggested that temperature itself is not the key factor influencing semen quality but temperature change may be.

Our results are consistent with the widely accepted concept that the duration of abstinence can influence semen quality (18, 25, 40, 41). Second repetition??

This is the first investigation on the semen quality of a large cohort of military personnel originally recruited from different geographical areas of China ~~on semen quality~~, conducted according to ~~the~~ WHO recommendations. The subjects, which comprise one type of internal migrants within China, had markedly lower sperm concentrations, counts and motility by reference to WHO recommendations (1999, 2010). (repetition) Furthermore, our study carried out among servicemen, with relatively high participation rates which effectually decreases the selection bias, compared with many other similar studies (17, 42, 43) based on clinic or hospital populations or sperm donor banks with participation rates less than 20%. (repetition???). (We can remove this sentence). Our study therefore has an important strength to detect the influences on semen quality when considering the environment and lifestyle changes. The study has important clinical significance on infertility management, fertility preservation, especially for ~~those~~ military

personnel ~~who are~~ resident in ~~a~~ high altitude environment with strong exposure to ~~the~~ UV light.

Limitations: We have no information on semen quality changes before and after the participants were enlisted, ~~however a~~.—A longitudinal study to answer this question is in progress. ~~Furthermore, T~~there ~~are~~ ~~are~~ ~~relatively~~ few comparable studies on male reproductive function and the relevant lifestyle influence on semen quality in China (or ‘normal’ ranges (ref23)..???; poorer diet post-conception, or during childhood/puberty, Great Leap Forward???). ~~and i~~It would be ~~helpful enlightening~~ if we could compare the results of the semen quality evaluations of the ~~Chinese~~ military personnel servicemen to the ~~with~~ data derived from ~~of~~ the non-migrant populations of the each local region in China [since the military servicemen share some features in common with the true migrant populations in terms of locality and the outdoor climate].

[In conclusion, the servicemen have significantly low normal proportionality of sperm concentration, count and motility by reference to WHO recommendations (1999, 2010), and they also appear to have lower semen quality when compared to the subjects recruited from the healthy general population as reported by the other studies in China. Repetition —Environmental changes such as food, living habits, climate and altitude may influence the semen quality, whereas age has no significant effect on semen parameters. Sperm concentration, sperm count and motility are all significantly lower in the autumn than summer. The average air temperature has less influence on the semen quality. Higher sperm concentration and total sperm count are observed among the subjects after 4-7 days of abstinence.] ([We can remove this para.](#))

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References

1. Carlsen E, Giwercman A, Keiding N, Skakkebaek N. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992;305: 609-13.
2. 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: 281-5.
3. Adamopoulos DA, Pappa A, Nicopoulou S, Andreou E, Karamertzanis M, Michopoulos J, et al. 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: 1936-41.
4. 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: 467-71.
5. Van Waeleghem K, De Clercq N, Vermeulen L, Schoonjans F, Comhaire J. Deterioration of sperm quality in young healthy Belgian men. *Hum Reprod* 1996;11: 325-9.
6. Gandini L, Lombardo F, Culasso F, Dondero F, Lenzi J. Myth and reality of the decline in semen quality: an example of the relativity of data interpretation. *J Endocrinol Invest* 2000;23: 402-11.
7. Paasch U, Thieme C, Glander HJ. Men born in the region of Leipzig (Saxony, Germany) between 1960 and 1970 showed a significantly decreased sperm count (examination of 3432 individuals). *Andrologia* 2003;35: 375-7.
8. Jørgensen N, Asklund C, Carlsen E, Skakkebaek NEJ. Coordinated European investigations of

- semen quality: results from studies of Scandinavian young men is a matter of concern. *Int J Androl* 2006;29: 54-61.
9. Suominen J, Vierula M. Semen quality of Finnish men. *BMJ* 1993;306: 1579.
 10. 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: 471-2.
 11. 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: 1009-14.
 12. Paulsen CA, Berman NG, Wang C. Data from men in greater Seattle area reveals no downward trend in semen quality: further evidence that deterioration of semen quality is not geographically uniform. *Fertil Steril* 1996;65: 1015-20.
 13. Berling S, Wolner-Hanssen P. No evidence of deteriorating semen quality among men in infertile relationships during the last decade: a study of males from Southern Sweden. *Hum Reprod* 1997;12: 1002-05.
 14. 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: 1059-64.
 15. Seo JT, Rha KH, Park YS, Lee MS. Semen quality over a 10-year period in 22,249 men in Korea. *Int J Androl* 2000;23: 194-8.
 16. 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: 11-7.
 17. Andersen AG, Jensen TK, Carlsen E, Jorgensen N, Andersson AM, Krarup T, et al. High frequency of sub-optimal semen quality in an unselected population of young men. *Hum Reprod* 2000;15: 366-72.

18. Jørgensen N, Carlsen E, Nermoen I, Punab M, Suominen J, Andersen AG, 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. Punab M, Zilaitiene B, Jorgensen N, Horte A, Matulevicius V, Peetsalu A, et al. Regional differences in semen qualities in the Baltic region. *Int J Androl* 2002;25: 243-52.
20. Lin DH, Fang XY, Li XM, Ling XY. A comparison of social adaptation among rural-to-urban migrants with different careers [in Chinese]. *Chn Mental Health J* 2007;21: 400-3.
21. IUSSP. *Multilingual Demographic Dictionary* 1982.
22. World Health Organization .WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction. In: *Collection and Examination of Human Semen*. Cambridge: Cambridge University Press 1999: 4-14.
23. Cooper TG, Noonan E, Eckardstein S, Jacques AG, Gordon Baker H, Hermann M, et al. World Health Organization reference values for human semen characteristics. *Hum Reprod* 2009;24: 231-45.
24. Li YF, Lin H, Ma M, Li LB, Cai M, Zhou NY, et al. Semen quality of 1346 healthy men, results from the Chongqing area of southwest China. *Hum Reprod* 2009;24: 459-69.
25. Gao J, Gao ES, Yang Q, Walker M, Wu JQ, et al. Semen quality in a residential, geographic and age representative sample of healthy Chinese men. *Hum Reprod* 2007;22: 477-84.
26. Liu M, Ma C, Tang L, Wen R, Deng S, Wang Q, et al. Quality analysis of the primary semen samples from 512 donors [in Chinese]. *Nat J Androl* 2004;10: 734-6.
27. Wu JQ, Yang QY, Tong JG, Yuang W, Bi LW, et al. Reference value of semen quality in Chinese young man. *Contraception* 2002;65: 365-8.
28. Jensen TK, Andersson AM, Jgensen N, Andersen AG, Carlsen E, Petersen JH, et al. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil*

Steril 2004;82: 863-70.

29. Auger J, Jouannet P. Evidence for regional differences of semen quality among fertile French men. *Hum Reprod* 1997;12: 740-45.
30. Yan SW, Tang J, Zhang N, Wang X, Lu H, Wang M. Investigation of Semen Quality of 18-35 Years Old Chinese Army Men [in Chinese]. *Nat J Androl* 2007;13:134-7.
31. Iwasaki A, Gagnon C. Formation of reaction oxygen species in spermatozoa of infertile patients. *Fertil Steril* 1992;57: 409-16.
32. Yu LK, Gui JH, Feng J, Hu B, Huang GX, et al. Comparison of sperm parameters between male adults at different altitudes. *Nat J Androl* 2007;13: 122-4.
33. Kidd SA, Eskenazi B, Wyrobek AJ. Effects of male age on semen quality and fertility: a review of the literature. *Fertil Steril* 2001;75: 237-48.
34. 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.
35. Eskenazi B, Wyrobek AJ, Slotter E, Kidd SA, Moore L, Young S, et al. The association of age and semen quality in healthy men. *Hum Reprod* 2003;18: 447-54.
36. 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: 942-9.
37. Politoff L, Birkhauser M, Almendral A, Zorn A. New data confirming a circannual rhythm in spermatogenesis. *Fertil Steril* 1989;52: 486-9.
38. 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: 277-81.
39. Levine RJ, Brown MH, Bell M, Shue F, Greenberg GN, et al. Air-conditioned environments do not prevent deterioration of human semen quality during the summer. *Fertil Steril* 1992;57:

1075-83.

40. Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, et al. Regional differences in semen quality in Europe. *Hum Reprod* 2001;16: 1012-19.
41. Levitas E, Lunenfeld E, Weiss N, Friger M, Har-Vardi I, Koifman A, et al. Relationship between the duration of sexual abstinence and semen quality: analysis of 9,489 semen samples. *Fertil Steril* 2005;83: 1680-6.
42. Lalos A, Daniels K, Gottlieb C, Lalos O. Recruitment and motivation of semen providers in Sweden. *Hum Reprod* 2003;18: 212-6.
43. Muller A, De La Rochebrochard E, Labbe-Decleves C, Jouannet P, Bujan L, Mieusset R, et al. Selection bias in semen studies due to self-selection of volunteers. *Hum Reprod* 2004;19: 2838-44.

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Table/figure legends

TABLE 1 Characteristics of the study population.

TABLE 2 Mean semen characteristics by areas.

TABLE 3 Mean semen characteristics by potential risk factors.

TABLE 4 Effects of potential risk factors on semen parameters.

Supplemental Figure 1. The locations of six selected districts on Chinese maps.

Supplemental Table 1. The characteristics of the six selected districts.