Int J Clin Exp Med 2016;9(3):6783-6788
www.ijcem.com /ISSN:1940-5901/IJCEM0018367

Original Article

Effect of high altitude exposure on spermatogenesis in male military personnel

Haixiang Hu[1*], Yajing Gao[2*], Hong Fang[1], Jing Sun[1], Lizhao Wei[1], Changcheng Li[1], Hongyuan Liu[1], Shaoqiang Xu[1], Haohao Ding[1]

[1]Department of Andrology, Chinese PLA Air Force General Hospital, Beijing 100142, China; [2]Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100029, China. [*]Equal contributors.

Received October 23, 2015; Accepted March 5, 2016; Epub March 15, 2016; Published March 30, 2016

Abstract: Objective: To investigate changes in semen quality of Chinese military servicemen at different altitudes. Study design: Randomized controlled study. Military servicemen aged between 18 and 25 years who had resided in a region for 2 to 3 years were recruited between 2008 and 2012. Main parameters included semen routine; semen volume (mL), sperm density (10 million/mL) and total sperm count (10 million); sperm vitality: grade A (%), grade A+B (%) and grade A+B+C (%). Results: The Ganbala group had the lowest semen volume (2.72 mL), which was significantly lower than that of the other 3 groups. The sperm density in the Ganbala group (33.89×10^6/mL) was markedly lower than that of the other three groups. The total sperm count was the lowest in the Golmud group (80.61×10^6). The percentage of grade A sperms in the Lhasa group (12.04%) and the Ganbala group (12.31%) was significantly lower than that of the Golmud group (18.63%) and Chengdu group (18.73%). The percentage of grade A+B sperms in the Lhasa group (24.50%) and the Ganbala group (24.93%) was significantly lower than that of the Golmud group (29.35%) and Chengdu group (30.54%). Conclusion High altitude (> 3650 m) has marked impact on semen quality.

Keywords: High altitude, military servicemen, semen parameters, semen quality

Introduction

Semen quality, as a clinical indicator of male reproductive health, is extensively used for the diagnosis and prognosis of reproductive disorders in males [1-3]. Multiple factors are known to impact on semen quality including temperature, climate, seasons, ethnicity and lifestyles [4-7], but great controversy still remains regarding the exact role of these factors in determining semen quality [8, 9]. Altitude is also known to impact on semen quality and altitudinal variations may contribute to differences in results on semen quality. An analysis of 58 papers published between 1955 and 2003 suggests that semen quality varies with altitudes, but the results are conflicting [10-14], which are likely due to differences in sampling method, differences in ethnicity and environmental differences. Therefore, it is necessary to define the impact of altitude on semen quality.

China is a vast country with significant variations in altitude, which provides a unique environment for investigating effects of different altitudes on semen quality. Our previous study demonstrated apparent effect of altitude on semen quality. Another study on the semen quality of military servicemen from six different regions of China showed that seasonal and altitudinal variations impacted on seamen quality while age, length of residence in a region and place of birth [15].

In the current study, we would like to understand more about the association of altitude variation and the quality of semen. The semen quality in different altitudes regarding semen volume, concentration, total count and mortality were examined. A large sample size of seamen was collected from 1053 Chinese military servicemen who have resided in one of the four regions of different altitudes in China for 2 to 3 years between 2008 and 2012.

Materials and methods

Experimental design

We recruited military servicemen from four regions of different altitudes in China: Ganbala (altitude: 5347 m), Lhasa (altitude: 3650 m), Golmud (altitude: 2800 m) and Chengdu (altitude: 700 m) between 2008 and 2012. Questionnaire management, physical examination and collection and analysis of semen specimens were performed by two groups of investigators at General Air Force Hospital, Beijing, China. The study protocol was approved by the local ethic committees at General Air Force Hospital and the 4 regions. All study participants provided written informed consent.

Study subjects

Totally 1200 military servicemen were screened and 147 (12.3%) military servicemen were excluded. Among them, 109 (9.1%) were excluded due to disease of the reproductive system, and 39 (3.2%) due to abstinence < 2 days or > 7 days. All subjects were Han Chinese and came from 21 provinces, with 532 from the South and 521 from the North. All subjects had resided in their current region for 2 to 3 years.

General survey

Data on age, date of birth, habits, length of military service, job, sex history, reproduction history, drug and alcohol use, a drug history of current and previous illness were recorded as analytical references.

Physical examination

All subjects received physical examination including genital examination by experienced urologists. Illnesses were ruled out by the physical examination criteria for new military servicemen. For this particular study, physical examination for secondary sexual traits was performed. Varices, hydrocele, orchiepididymitis and other diseases of the reproductive and urinary system were excluded.

Semen analysis

Semen specimens were analyzed according to the 1999 WHO recommendations. Semen quality analysis was carried out after liquidation for 1 h after collection.

Statistical analyses

Continuous data are summarized as mean ± standard deviation (SD), while categorical data are summarized as numbers and percentages. Demographic characteristics were compared among four groups by one-way analysis of variance (ANOVA), and by Chi-square test for categorical data, as appropriate.

Nevertheless, as semen parameters follow markedly skewed (non-normal) distributions, unadjusted mean and median values, standard deviation (SD), and interquartile range (IQR, 25^{th} to 75^{th} percentiles) as well were calculated for each variable. A nonparametric Kruskal-Wallis test was used to compare between-group medians of semen parameters. The relationships between the semen parameters and the altitudes of region were further investigated by nonparametric Spearman's rank correlation coefficients. Moreover, percentages coincident with WHO recommendations 1999 were also calculated and stratified according to military service location. A Cochran-Armitage test for trend was applied to investigate whether normal semen percentages increased across regions with lowered altitudes. All statistical analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC). A two-tailed $P < 0.05$ was taken to indicate statistical significance.

Results

Baseline characteristics of study population

The baseline characteristics of 998 men are summarized in **Table 1**. A total of 185 participants were recruited from Ganbala (altitude: 5347 m), 282 from Lhasa (altitude: 3650 m), 261 from Golmud (altitude: 2800 m), and 270 from Chengdu (altitude: 700 m), respectively. Over 40% of participants aged 24 years or more (mean: 22.5 years, range: 18 to 26 years). All participants had at least two years in military service (mean: 2.48 years). The mean ejaculation abstinence time was 4.7 days (range: 3 to 7 days), and average time to start of semen analysis was 33 minutes (range: 20 to 100 minutes). The baseline characteristics with regard to age, time in military service, birth place, marriage status, whether having children, smoking and alcohol consumption, ejaculation abstinence time as well as time to start of semen analysis did not differed significantly among participants from 4 different regions (all $P > 0.05$; **Table 1**).

Characteristics of semen parameters

The semen characteristics of the 998 men are listed in **Table 2**. The significant differences in median values were found for all semen param-

Table 1. Characteristics of the study population, according to 4 representative regions of different altitudes (N = 998)

Characteristics	Ganbala (5347 m)	Lhasa (3650 m)	Golmud (2800 m)	Chengdu (700 m)	*P*-value
Number of participants	185	282	261	270	
Mean age (year)[1]	22.5 ± 2.4	22.6 ± 2.4	22.5 ± 2.5	22.3 ± 2.6	0.444
Age (year)[2]					
18-20	52 (28.1)	66 (23.4)	75 (28.7)	92 (34.1)	0.142
21-23	54 (29.2)	90 (31.9)	70 (26.8)	63 (23.3)	
24+	79 (42.7)	126 (44.7)	116 (44.4)	115 (42.6)	
Mean time in military service (year)[1]	2.49 ± 0.50	2.47 ± 0.50	2.48 ± 0.50	2.48 ± 0.50	0.989
Time in military service (year)[2]					
2	95 (51.4)	149 (52.8)	137 (52.5)	140 (51.9)	0.980
3	90 (48.7)	133 (47.2)	124 (47.5)	130 (48.2)	
Birth place[2]					
North China	101 (54.6)	152 (53.9)	141 (54.0)	143 (53.0)	0.988
South China	84 (45.4)	130 (46.1)	120 (46.0)	127 (47.0)	
Marriage status[2]					
Single	83 (44.9)	130 (46.1)	120 (46.0)	125 (46.3)	0.992
Married	102 (55.1)	152 (53.9)	141 (54.0)	145 (53.7)	
Having Children[2]					
No	84 (45.4)	130 (46.1)	120 (46.0)	125 (46.3)	0.998
Yes	101 (54.6)	152 (53.9)	141 (54.0)	145 (53.7)	
Smoking and alcohol consumption[2]					
No	84 (45.4)	130 (46.1)	121 (46.4)	124 (45.9)	0.998
Yes	101 (54.6)	152 (53.9)	140 (53.6)	146 (54.1)	
Mean ejaculation abstinence time (day)[1]	4.8 ± 1.0	4.7 ± 1.1	4.6 ± 0.9	4.8 ± 0.9	0.315
Mean time to start of semen analysis (min)[1]	33.6 ± 9.3	32.4 ± 10.9	33.1 ± 7.7	33.2 ± 7.8	0.551

[1]Continuous data are presented as mean ± standard deviation (SD) and compared among 4 groups by ANOVA; [2]Categorical data are expressed as numbers (%) and compared among 4 groups by Chi-square test.

eters among participants from 4 different regions (Kruskcal-Wallits test, all $P < 0.05$; **Table 2**). The participants based in Ganbala had the lowest median semen volume (2.5 mL), while those based in Chengdu had the highest (4.0 mL). The participants based in Golmud had the lowest median sperm concentration (72×10^6/mL), while those based in Lhasa had the highest (110×10^6/mL). Regarding total sperm count, those based in Golmud had the highest median value (37.7×10^6) while those based in Ganbala had the lowest (26.1×10^6). The highest median value of sperm rapid progressive motility was found in those based in Golmud (14.3%) and the lowest found in those based in Lhasa (7.9%). Regarding sperm progressive motility and total mobility as well, the median values were 20.6% and 29.3% for those based in Ganbala, 20.8% and 33.9% for those based in Lhasa, 27.6% and 38.6% for those based in Golmud, and 27.8% and 41.7% for those based in Chengdu, respectively, which showing an inverse relationship between sperm motility and altitude (Spearman's rank correlation coefficients: -0.193 and -0.151 for sperm progressive motility and total mobility, respectively, both $P < 0.001$).

Normal percentage of semen parameters according to 1999 WHO standards

The normal percentages of semen parameters defined by the WHO recommendations (1999) are listed in **Table 3**, stratified by 4 representative regions of different altitudes. With regard to semen volume, sperm concentration as well as total sperm count, the normal percentages based on 1999 WHO standards did not show a significant increasing trend across regions with sequentially lowered altitudes (Ganbala: 5347 m, Lhasa: 3650 m, Golmud: 2800 m, Chengdu: 700 m). Nevertheless, the normal percentages

Table 2. Characteristics of semen parameters of study participant, according to 4 representative regions of different altitudes (N = 998)

Characteristics of semen parameters	Ganbala (5347 m)	Lhasa (3650 m)	Golmud (2800 m)	Chengdu (700 m)	*P*-value†
Semen volume (mL)					
Mean ± SD	2.8 ± 1.0	3.0 ± 0.8	3.0 ± 0.9	3.2 ± 1.0	
Median (IQR)	2.5 (2.0, 4.0)	3.0 (2.5, 4.0)	3.0 (2.0, 4.0)	4.0 (3.0, 4.0)	< 0.001***
Sperm concentration ($\times 10^6$/mL)					
Mean ± SD	118.0 ± 97.8	157.3 ± 139.2	80.6 ± 67.7	95.5 ± 63.3	
Median (IQR)	94.0 (53.0, 156.0)	110.0 (63.0, 212.0)	72.0 (29.0, 118.0)	83.0 (51.0, 121.0)	< 0.001***
Total sperm count ($\times 10^6$)					
Mean ± SD	33.3 ± 26.4	38.8 ± 29.6	40.6 ± 30.0	35.3 ± 27.2	
Median (IQR)	26.1 (14.9, 43.7)	26.5 (18.3, 49.0)	37.7 (15.9, 56.0)	31.2 (19.0, 49.6)	0.046*
Sperm rapid progressive motility (A%)					
Mean ± SD	12.2 ± 11.5	12.0 ± 10.5	18.6 ± 12.7	18.7 ± 14.0	
Median (IQR)	8.3 (4.8, 16.7)	7.9 (3.8, 19.3)	14.3 (8.3, 30.2)	12.9 (6.3, 31.8)	< 0.001***
Sperm progressive motility [(A+B)%]					
Mean ± SD	24.3 ± 16.3	24.5 ± 15.8	29.3 ± 14.3	30.5 ± 15.7	
Median (IQR)	20.6 (12.6, 34.2)	20.8 (11.8, 37.3)	27.6 (17.8, 41.9)	27.8 (16.8, 44.1)	< 0.001***
Total motility [(A+B+C)%]					
Mean ± SD	34.3 ± 20.4	35.4 ± 22.1	39.5 ± 18.4	39.9 ± 15.9	
Median (IQR)	29.3 (18.6, 45.2)	33.9 (16.7, 51.0)	38.6 (26.4, 51.2)	41.7 (27.9, 51.6)	< 0.001***

†The *P*-values were derived by non-parametric Kruskal-Wallis test to compare intergroup median values. Statistically significant: *P < 0.05, **P < 0.01, ***P < 0.001.

according to 1999 WHO standards for those based in Ganbala, Lhasa, Golmud, and Chengdu were 11.9%, 14.9%, 28.0%, and 38.5% with regard to sperm rapid progressive motility (*P*-value for trend < 0.001), and 7.0%, 6.4%, 10.3%, and 14.1% with regard to sperm progressive motility (*P*-value for trend = 0.002), both indicating the qualified sperm rapid progressive/progressive motility might be lowered as the altitudes elevated.

Discussion

Altitudinal variations may contribute to differences in semen quality, but the results so far are conflicting [11-14]. This is possibly due to the presence of compounding factors and differences in analytical methods.

In the current study, we exclusively focused on the effect of altitudinal variations by investigating young military service men from 4 regions of different altitudes in China. These subjects from different regions were comparable in demographic characteristics. The altitude studied ranges from 700 m to 5347 m. The current study excluded other possible determinants of semen quality and only studied the effect of altitude on semen quality and found that altitude impacted on semen. Noticeable effect on semen was observed at altitude above 3650 m and no apparent effect was observed at altitude below 2800 m. We found that the semen volume and sperm density in the Ganbala group (altitude: 5347 m) were marked lower than other three groups (*P* < 0.01). The total sperm count in the Ganbala group and Lhasa group (altitude: 3650 m) was markedly higher than that of Golmud group (altitude: 2800 m) and Chengdu group (altitude: 800 m) (*P* < 0.01). The percentage of grade A sperms, grade A+B sperms, or grade A+B+C sperms in the Lhasa group and the Ganbala group was also significantly lower than that of the Golmud group and the Chengdu group. The semen quality was lower than that reported by other studies in China [8, 16]. One difference is that the study participants in the current study were younger in age and had worked for some time in high altitude.

The current results were compared to the 1999 Semen Quality Guideline, the semen volume, sperm density and total sperm count was 86.69%, 66.19%, and 79.13%, respectively. The rate of normal grade A sperms in the Ganbala group (22.87%) and the Lhasa group (24.89%) was markedly lower than that of the Golmud group (35.97%) and the Chengdu group (38.52%). The rate of normal grade A+B sperms in the Ganbala group (21.78%) and the Lhasa

Table 3. Normal percentage of semen parameters (1999 WHO standards), according to 4 representative regions of different altitudes (N = 998)

1999 WHO Semen standards	Ganbala (5347 m)	Lhasa (3650 m)	Golmud (2800 m)	Chengdu (700 m)	*P*-value for Trend‡
Semen volume (mL)					
Abnormal (< 2 mL)†	21 (11.4)	24 (8.5)	24 (9.2)	30 (11.1)	0.852
Normal (≥ 2 mL)	164 (88.7)	258 (91.5)	237 (90.8)	240 (88.9)	
Sperm concentration ($\times 10^6$/mL)					
Abnormal (< 20×10^6/mL)†	65 (35.1)	90 (31.9)	84 (32.2)	72 (26.7)	0.065
Normal (≥ 20×10^6/mL)	120 (64.9)	192 (68.1)	177 (67.8)	198 (73.3)	
Total sperm count ($\times 10^6$)					
Abnormal (< 40×10^6)†	26 (14.1)	30 (10.6)	91 (34.9)	35 (13.0)	0.083
Normal (≥ 40×10^6)	159 (86.0)	252 (89.4)	170 (65.1)	235 (87.0)	
Sperm rapid progressive motility (A%)					
Abnormal (< 25%)†	163 (88.1)	240 (85.1)	188 (72.0)	166 (61.5)	< 0.001***
Normal (≥ 25%)	22 (11.9)	42 (14.9)	73 (28.0)	104 (38.5)	
Sperm progressive motility [(A+B)%]					
Abnormal (< 50%)†	172 (93.0)	264 (93.6)	234 (89.7)	232 (85.9)	0.002**
Normal (≥ 50%)	13 (7.0)	18 (6.4)	27 (10.3)	38 (14.1)	

†Abnormal values of semen parameters were defined by the WHO recommendations (1999). The 1999 WHO standards: semen volume < 2 mL, sperm concentration < 20×10^6/mL, sperm total count < 40×10^6, sperm rapid progressive motility < 25% and sperm progressive motility < 50%. ‡The *P*-values were determined by Cochran-Armitage test for trend to investigate whether normal semen percentages increased across regions with decreasing altitudes. Statistically significant: *P < 0.05, **P < 0.01, ***P < 0.001.

group (25.16%) was significantly lower than that of the Golmud group (40.23%) and Chengdu group (42.40%).

It was obviously that sperm quality is influenced by multiple factors and semen quality will change when the body is subject to influences by the external environment [17]. For example, high altitude is marked by low temperature, a hypoxic environment, a low atmosphere, and high solar irradiation. Previous studies reported that the rarefied environment at high altitude lowers testosterone levels [13-15, 18, 19]. Possible mechanisms include (1) hypoxia suppresses the function of male sexual glands by directly influencing sperm quality and causing elevations of sex hormone binding proteins, which indirectly reduce free testosterone; (2) hypoxia causes damages to the cortex of the adrenal gland and interstitial cells in the testes, directly reducing the secretion of testosterone. Meanwhile, the substrate for synthesis of estradiol is reduced, indirectly reducing the amount of estradiol. In addition, low estradiol levels could lower airway responsiveness, increase the tidal volume and impact on oxygen exchange, thus aggravating hypoxia. The body mounts a stress response to the hypoxic environment. Microvessels and tissues under stress show changes in physiology and morphology, thereby indirectly influencing the endocrine function, causing changes in sperm quality. Severe hypoxia reduces the secretion of gonadotropin-releasing hormone (GRH) from the hypothalamus. The hypothalamus-pituitary gland-sex gland axis is the key to the initiation of reproduction. Reduced secretion of GRH in males directly lowers semen quality [20].

The current study excluded age, time residing in high altitude, time for sexual abstinence and season as confounding factors for semen quality. By focusing on an exclusive group of population (military servicemen), we found that except the semen volume, all other parameters were lower than those reported for other large sample studies in other countries on semen quality of normal males, especially in the Ganbala group and Lhasa group [7, 11, 13, 17, 18]. We also noticed the total sperm count at altitude above 3650 m was higher than that of the Golmud and Chengdu group, while vitality and viability rate was lower at high altitude. We speculate that the increased sperm count in high altitude may be due to adaptive or compensatory response of the host in response to the rarified environment in high altitude. Therefore, further studies are required to elucidate the mechanism underlying changes in semen quality at high altitude. In conclusion, high altitude (> 3650 m) has marked impact on semen quality.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Haixiang Hu, Department of Andrology, Chinese PLA Air Force General Hospital, 30 Fucheng Road, Haidian District, Beijing 100142, China. Tel: +86-15611161097; E-mail: hhx.afgh@gmail.com

References

[1] Cooper TG and Yeung CH. Computer-aided evaluation of assessment of "grade a" spermatozoa by experienced technicians. Fertil Steril 2006; 85: 220-224.

[2] Fuse H, Akashi T, Nozaki T, Nishio R and Mizuno I. Assessment of sperm quality analyzer II B: comparison with manual semen analysis and CASA. Arch Androl 2005; 51: 65-67.

[3] Zhang H, Cai SL and Yu JJ. [A comparative study of computer assistant sperm analysis with rontine sperm analysis]. Zhejiang Da Xue Xue Bao Yi Xue Ban 2002; 31: 215-218.

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

[5] Carlsen E, Petersen JH, Andersson AM and Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. Fertil Steril 2004; 82: 358-366.

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

[7] Muthusami KR and Chinnaswamy P. Effect of chronic alcoholism on male fertility hormones and semen quality. Fertil Steril 2005; 84: 919-924.

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

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

[10] Garcia-Hjarles MA. [Sperm count and seminal biochemistry of high altitude inhabitants and patients with chronic altitude sickness]. Arch Biol Med Exp (Santiago) 1989; 22: 61-67.

[11] Sawhney RC, Chhabra PC, Malhotra AS, Singh T, Riar SS and Rai RM. Hormone profiles at high altitude in man. Andrologia 1985; 17: 178-184.

[12] Karvonen J, Peltola E, Saarela J and Nieminen MM. Changes in running speed, blood lactic acid concentration and hormone balance during sprint training performed at an altitude of 1860 metres. J Sports Med Phys Fitness 1990; 30: 122-126.

[13] Okumura A, Fuse H, Kawauchi Y, Mizuno I and Akashi T. Changes in male reproductive function after high altitude mountaineering. High Alt Med Biol 2003; 4: 349-353.

[14] Savourey G, Garcia N, Caravel JP, Gharib C, Pouzeratte N, Martin S and Bittel J. Pre-adaptation, adaptation and de-adaptation to high altitude in humans: hormonal and biochemical changes at sea level. Eur J Appl Physiol Occup Physiol 1998; 77: 37-43.

[15] Zou Z, Hu H, Song M, Shen Y, Guo X, McElreavey K, Bittles AH and Wang W. Semen quality analysis of military personnel from six geographical areas of the People's Republic of China. Fertil Steril 2011; 95: 2018-2023, 2023. e2011-2013.

[16] Li G, Huang P, Ma HZ, Ge L, Xie Y and Wan QZ. [Investigation on the sperm quality of 549 college students in Chengdu area]. Zhonghua Nan Ke Xue 2003; 9: 673-675, 678.

[17] Toft G, Pedersen HS, Bonde JP and Team IR. Semen quality in Greenland. Int J Circumpolar Health 2004; 63 Suppl 2: 174-178.

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

[19] Escudero F, Gonzales GF and Gonez C. Hormone profile during the menstrual cycle at high altitude. Int J Gynaecol Obstet 1996; 55: 49-58.

[20] Fenster L, Katz DF, Wyrobek AJ, Pieper C, Rempel DM, Oman D and Swan SH. Effects of psychological stress on human semen quality. J Androl 1997; 18: 194-202.