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Chronic hypoxia and male fertility
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·*Original Article*·

Evidence that chronic hypoxia causes reversible impairment on male fertility

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Abstract

Aim: To evaluate the effect of chronic hypoxia on human spermatogenic parameters and their recovery time. **Methods:** Seminological parameters of six male healthy mountain trekkers were evaluated in normoxia at sea level. After 26 days exposure to altitude (ranging from 2 000 m to 5 600 m, Karakorum Expedition) the same parameters were again evaluated after returning to sea level. These parameters were once again evaluated after 1 month and then again after 6 months. **Results:** Sperm count was found to be lower immediately after returning to sea level (P = 0.0004) and again after a month (P = 0.0008). Normal levels were reached after 6 months. Spermatic motility (%) shows no reduction immediately after returning to sea level (P = 0.0583), whereas after 1 month this reduction was significant (P = 0.0066). After 6 months there was a recovery to pre-hypoxic exposure values. Abnormal or immature spermatozoa (%) increased immediately after returning to sea level (P = 0.0067) and then again after 1 month (P = 0.0004). After 6 months there was a complete recovery to initial values. The total number of motile sperm in the ejaculate was found to be lower immediately after returning to sea level (P = 0.0024) and then again after 1 month (P = 0.0021). After 6 months there was a recovery to pre-hypoxic exposure values. **Conclusion:** Chronic hypoxia induces a state of oligospermia and the normalization of such seminological parameters at the restoration of previous normoxic conditions after 6 months indicate the influence of oxygen supply in physiological mechanisms of spermatogenesis and male fertility. *(Asian J Androl 2008 Jul; 10: 602–606)*

Keywords: male fertility; hypoxia; seminological parameters; high altitude

1 Introduction

High altitude hypoxia has been known to influence male fertility. At the end of the 16th century, Spanish conquistadores, who settled in Bolivia at Potosi (4 267 m above sea level), only managed to conceive children with

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Spanish blood 53 years after the foundation of the city. Furthermore, the capital of Peru was moved from Jauia to Lima, at sea level, because of the incapacity of imported animals to reproduce. Jauia (3 500 meters above sea level) was considered "a sterile place", where horses, pigs and fowls could not be raised, whereas 100 years later it was a principle pig and poultry producing area supplying Lima with these products [1,2].

In the present paper, we discuss the effects of chronic hypoxia on male reproductive functions. Few published studies consider the physiological and physiopathological effects of chronic hypoxia on male fertility specifically.

It is well known that at high altitudes, haemoglobin carries less oxygen. This occurs because the partial inspired pressure of oxygen decreases and the amount of oxygen available for diffusion into the bloodstream decreases. The resulting hypoxemia stresses oxygen-dependent metabolic processes throughout the organism [3, 4]. This oxygen reduction induces a reversible spermatogenic dysfunction [5]. Despite high altitudes, populations have been reproducing for thousands of years, and mean total fertility values in areas of high altitudes are comparable to respective mean values in whole populations. However, subjects from sea level seem to have difficulty reproducing at high altitudes, especially if they are Caucasian. Cattle and other animals sometimes fail to reproduce. This can only be avoided after cross-breeding with acclimatized strains after several generations [6].

High altitude exposure affects spermatogenesis, particularly the onset of mitosis and spermiation [7]. Spermatogenesis is the process by which germ stem cells develop into mature spermatozoa. It is perhaps one of the most important and delicate processes that occurs in the male body and is essential for sexual reproduction. In the animal model, it has been shown that hypoxia exposure induces a partially reversible decrease in semen volume, sperm count and sperm motility [8]. Also, semen analyses of the members of the Masherbrum expedition (7 821 m above sea level) showed a reversible sperm count decrease, an increase in abnormally shaped sperm and showed no change in semen volume [9]. In 1982, Bustos-Obregon and Olivares [10] described damage in mature spermatozoa after hypoxia exposure. Histological examination of rat testis after hypoxia show changes in testicular morphology, loss of spermatogenic cells in all stages of the spermatogenic cycle, degeneration of the germinal epithelium and spermatogenic arrest, degeneration and sloughing of spermatogenic cells in occasional tubules and differences in the volume of the testis occupied by Leydig cells [11, 12]. These changes are associated with an increase in interstitial space and in testicular mass, a decrease in height of the seminiferous epithelium, depletion of cellular elements and vacuolization in epithelial cells and folding of the basal membrane [11]. After experimental acute hypoxia, the number of spermatogenic epithelial cells, Sertoli cells and Leydig cells in testicular tissue reversibly decrease [13]. The aim of the present work is to evaluate the effect of chronic hypoxia on human spermatogenic parameters and their time recovery.

2 Materials and methods

Before altitude exposure, the mean value of seminological parameters of six male healthy mountain trekkers (average age 45 years, ranging from 32–71 years) were evaluated in normoxic conditions at sea level. After 26 days of exposure to altitude (ranging from 2 000 m to 5 600 m above sea level, Karakorum Expedition) the values of seminological parameters were evaluated after the subjects returned to sea level. Furthermore, the mean values of seminological parameters were once again evaluated after 1 month and then again after 6 months.

The study was carried out in accordance with the Bioethical Committee of the University of Chieti and in accordance with the Declaration of Helsinki (as revised in Edinburgh in 2000). All semen samples were analyzed in the same laboratory according to standardized methods throughout the study period. Samples were collected by masturbation into wide-mouth glass containers after 3 days of sexual abstinence. Informed consent was obtained from all subjects. The analyzed variables were the seminal fluid volume (mL), sperm count ($\times 10^6$ sperm/mL of ejaculate), motility (percentage of moving spermatozoa), sperm mobility according to Hotchkiss, vitality index (percentage of mobile nemasperms after 2 h), percentage of sperm with abnormal or immature morphology (spermocytogram) and the total number of motile sperm in the ejaculate (for each patient semen specimen was calculated as = volume × concentration × progressive motility).

To be included in the present study, the spermiogram had to be normal according to the standard criteria of the World Health Organization (WHO) [14]. The following

criteria was requested for inclusion in the study: good health, negative history for pathologies compromising fertility and negative cultured sperm. Statistical analysis SPSS 10.0 software (SPSS, Chicago, IL, USA) using non-parametric statistic tests for coupled data (Wilcoxon test) was used. $P < 0.05$ was considered statistically significant.

3 Results

The mean values ± SD of seminological parameters are shown in Table 1. The mean value of sperm count ($\times 10^6$/mL) was found to be significantly reduced immediately after return to sea level (with respect to the normal value before hypoxic exposure): from 52.67 ± 18.30 to 18.85 ± 15.86 ($P = 0.0004$). After 1 month, sperm count showed a further decrease 17.55 ± 16.41 ($P = 0.0008$), returning to normal levels after 6 months 53.00 ± 8.72 (the reference value proposed by the WHO is 20×10^6/mL: oligospermic specimens revealed concentrations of less than 20×10^6 and normospermic specimens contained more than 20×10^6). The mean value of sperm motility percentage immediately after return to sea level was not significantly different, from 56.67 ± 16.33 to 45.01 ± 13.78 ($P = 0.0583$). After 1 month, this reduction showed a statistical significance of 36.67 ± 7.45 ($P = 0.0066$), going back to normal values after 6 months: 58.33 ± 12.13 (according to WHO recommendations, asthenozoospermia was assigned to semen samples with < 50% progressively motile spermatozoa a + b). The mean value of abnormal or immature spermatozoa percentage increases significantly immediately after return to sea level:31.67 ± 5.16 to 44.17 ± 9.17 ($P = 0.0067$). After a month, levels of abnormal or immature spermatozoa percentages showed a further increase, 46.67 ± 4.71 ($P = 0.0004$), returning to normal values after 6 months, 27.50 ± 10.63 ($P = 0.00422$) (WHO criteria: > 30% normal forms/100 cells evaluated). Calculating the mean value of the total number of motile sperm in the ejaculate, we found a significant reduction im-

Table 1. The mean values ± SD of seminological parameters before and after chronic hypoxia exposure.

Seminological parameters (means ± SD)	Before	Immediately after return	1 month after	6 months after
Sperm count ($\times 10^6$/mL)	52.67 ± 18.30	18.85 ± 15.86	17.55 ± 16.41	53.00 ± 8.72
Sperm motility (%)	56.67 ± 16.33	45.01 ± 13.78	36.67 ± 7.45	58.33 ± 12.13
Abnormal or immature spermatozoa (%)	31.67 ± 5.16	44.17 ± 9.17	46.67 ± 4.71	27.50 ± 10.63
Total number of motile sperm ($\times 10^6$)	58.63 ± 22.18	15.06 ± 15.87	12.88 ± 10.49	70.66 ± 25.20

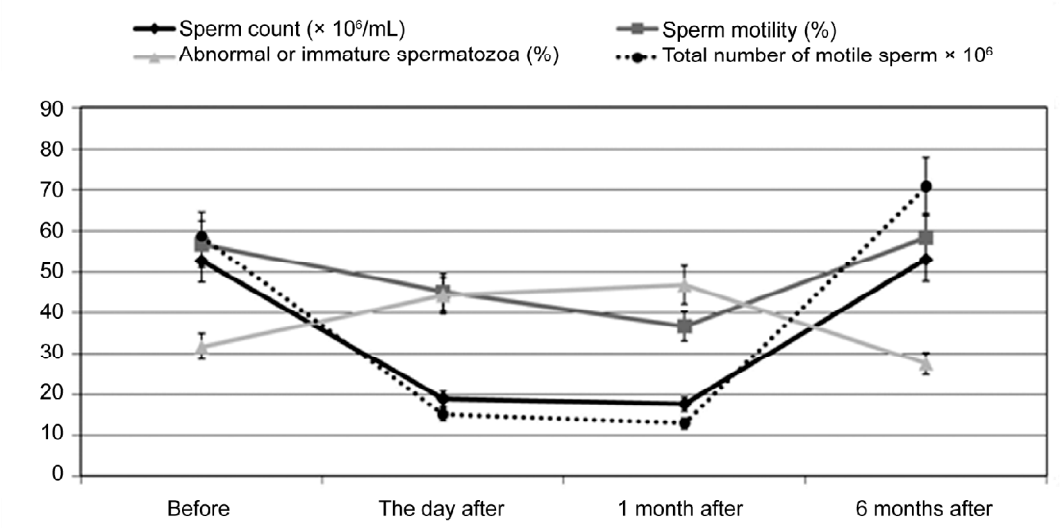

Figure 1. Effect of chronic hypoxia on human spermatogenic parameters and their time recovery.

mediately after subjects returned to sea level (with respect to values before hypoxic exposure): (58.63 ± 22.18) × 10^6 to (15.06 ± 15.87) × 10^6 (P = 0.0024). After 1 month the total number of motile sperm in the ejaculate showed a further decrease, (12.88 ± 10.49) × 10^6 (P = 0.0021), returning to normal levels after 6 months, (70.66 ± 25.20) × 10^6. A negative correlation between chronic hypoxia and seminological parameter values and their time recovery after the restoration of previous normoxic conditions is shown in Figure 1. No significant differences were shown in sperm volume (normal ejaculate volume is between 2 mL and 6 mL), mobility degree and vitality index [14].

4 Discussion

Organisms at high altitudes must adapt to the stress of limited oxygen availability in comparison to sea level and still sustain aerobic metabolic processes. At an altitude of 4 000 m, 1 L of air contains just 63% of the number of oxygen molecules present at sea level. Nevertheless, oxygen-requiring physiological processes must be maintained. The homeostatic processes that enable oxygen delivery under stress come from the evolution of natural selection in the sea level ancestral population, the high-altitude colonizing population or both [15].

High altitude chronic hypoxia induces negative effects on male fertility in individuals living at sea level, compared to those living at higher altitudes for many generations. Of course, when we say "chronic hypoxia" we have to specify if we consider an "intermittent" situation, minutes, hours, days, weeks or months of hypoxia exposure; for these reasons we consider a state of chronic hypoxia to be a condition of a reduction of oxygen supply for up to 6 h, whereas we consider a state of "acute hypoxia" to be an hypoxia exposure lasting from a few seconds to a few minutes [16].

Experimental and clinical evidence suggests mechanisms by which such adaptation is possible through natural selection and developmental processes. The present study demonstrated that oxygen reduction, as a result of exposure to chronic high altitude hypoxia, contributes to spermatogenesis and male fertility.

The mechanisms responsible for the hypoxic-damage in spermatogenesis are not fully understood. Regarding the effect of heat on the number and motility of spermatozoa, Setchell suggests that the heated testis is probably hypoxic and that damage might be caused not so much by the hypoxia directly, but by the generation of reactive oxygen species (ROS) [17]. In fact, evidence has accumulated supporting the pivotal role of ROS in the pathogenesis of many reproductive processes. ROS production is regulated by oxygen tension and under hypoxic conditions an increase in ROS has been reported, which can lead to a variety of intracellular effects. Oxidative stress attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. ROS induced DNA damage might accelerate the process of germ cell apoptosis, leading to the decline in sperm count associated with male infertility [18, 19].

Nevertheless, even if we did not measure ROS in the present study, our previous data suggest that exposure to stressful stimulus, such as chronic hyperoxia, might cause peroxidation damage in neonatal and old rat testis, but this toxic effect is not evident in young adult rat testis [20]. Evidently, mammals have evolved several mechanisms to minimize ROS-induced damage and the response of spermatogenic cells is linked to antioxidant systems [21] that seem to be more efficient in young adult rats. However, further experimentation is required to consider these results of value. There are few studies that contemplate the role of oxygen in the physiopathology of male infertility and it is clear that high altitude studies regarding reproduction cannot employ large samples, which results in some incongruence in the data seen in literature.

In conclusion, the negative influence of hypoxia on seminological parameters induces a state of oligospermia with reduced motility, a reduction of the total number of motile sperm and an increase in abnormal or immature spermatozoa. Values for sperm concentration, motility and morphology can be used to classify men as subfertile, of indeterminate fertility or fertile. Even if none of these measures are truly diagnostic of infertility [22], the consequent normalization of such seminological parameters after 6 months indicates a key role of oxygen supply in physiological mechanisms of spermatogenesis and in male fertility.

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