Low altitude return ameliorates semen parameters after high altitude exposure

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ABSTRACT

Background: A widely studied model of hypoxia is represented by high altitude (HA). Hence, HA hypoxia (HAH) is a challenge for people residing in or visiting high altitudes (Young and Reeves, 2002). Adaptation to HAH affects the homeostasis of several organs and the endocrine and metabolic functions. The aim of this study was to investigate the effect of HAH on the rat's semen and oxidative stress parameters.

Methods: This experimental study was carried out at Abha city, Saudi Arabia, high altitude, 2,800 m above sea level; Jazan city, 43 m above sea level, low altitude. A total of 72 rats were used in this study—8 rats as control; groups 1, 2, and 3 each of 8 rats and group 4 of 40 rats were kept at high altitude for 8, 16, 24, and 32 days, respectively. From group 4, 32 rats were taken to low altitude to testify the reversibility of the semen parameters.

Results: There were significant gradual decreases in the number and motility of the epididymal sperms in groups of rats exposed to HA during the first 3 weeks of HA exposure (HG1-HG3) with a maximum decreases to be seen in HG3 (−57.3 and −39.1%, respectively). However, the sperm count started to recover gradually on week 4 of HA exposure (HG4) and during all the periods of the reversal protocol achieved by returning the rats to the LA area (RG1-RG4). The maximum improvement in the sperm count and motility was seen in RG3 and RG4 which were not significantly different when compared with each other. The ANOVA test revealed that, in spite of the improvement in the sperm count which reach (109.3 ± 6.057 and 113.9 ± 8.967) in RG3 and RG4, their levels remained significantly low as those obtained in the control LA rats (129.2 ± 11.67).

Conclusion: Exposure of rats to hypoxia resulted in a decrease in the sperm count and motility and an increase in the sperm morphological abnormalities. To conclude, the current study showed that the adverse effect of hypobaric hypoxia on semen parameters is transient and reversible.

Keywords: Fertility, high altitude, hypoxia, rats

Introduction

A widely studied model of hypoxia is represented by high altitude (HA). Hence, HA hypoxia (HAH) is a challenge for people residing in or visiting high altitudes. The adaptation to HAH affects the homeostasis of several organs and the endocrine and metabolic functions.[1] Among the physiological effects of hypoxia, it has been suggested that newcomers from low-altitude (LA) areas have difficulties in fertility at HA; although, the fertility rates in native residents at HA is not lower than in populations at sea level (Gonzales 2007). Previous studies indicated exposure to HAH reduce the fertility and spermatogenesis of male rats (Cikutovic M., 2009). Several mechanisms including increase in testicular temperature, vascularisations and generation of testicular and sperm reactive oxygen species (ROS) and lipid peroxidation have been reported to play an essential roles in the deteriorious effect of hypobaric hypoxia (HH) on male fertility and poor sperm parameters (Cummins JM et al., 1994; Desai N, et al., 2010; Donayre J., 1966).[2]

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It was reported that the effect of a reduced spermatogenesis due to HH is associated with an increased vascularization and ROS production in the testis (Farias et al., 2010). The main sources of seminal ROS are immature spermatozoa and leukocytes (Farias et al., 2005). Elevated levels of ROS correlate negatively with sperm concentration and motility (Farias et al., 2008). Although several studies investigated the male reproductive function and the poor semen parameters at different altitudes, but most of them have utilized high altitude laboratories and/or simulated hypobaric chambers. Furtherly, most of these studies utilized only short time exposure of HA hypoxia and didn’t investigate the recovery after returning to LA areas or to the sea level (Farias et al., 2010, Farias et al. 2005).

There are few studies comparing semen parameters with higher and lower altitudes after ascending HA and descending to LA areas. Also, no study up to our knowledge is available to demonstrate such effect in the HA areas of the Kingdom of Saudi Arabia. This prompts us to thoroughly investigate the changes in semen and parameters over different time intervals of HA exposure using non-acclimatized native LA rats in a natural hypoxic environment provided by the high altitude locations of our laboratories which are located at mountains of Asser region (Saudi Arabia, 3150 m above sea level). The time dependent for recovery in the levels of these parameters after returning to LA areas was also investigated.

Materials and Methods

Experimental design
A total number of 72 adult male Wistar rats (200-250 g) were used. The experiment was approved by the Ethical Committee, college of Medicine, King Khalid University (Abha, Saudi Arabia). All animals were bred and maintained in the animal house of the College of Medicine at Jazan University (Jazan City, KSA).

The animals were divided into control group and experimental group. The control group was kept at LA (Jazan city). The experimental group transferred to HA (Abha city, College of medicine, KKU) and served as hypoxic group. The hypoxic group subdivided into eight groups. Samples were collected from group 1-4 weekly (Hyppoxy group), the remaining groups 5-8 retimed to LA (Reversal group) and samples were collected with same manner.

Tissue collections and preparation of homogenates
All rats were killed by an overdose of anaesthetic agent (phenobarbital, 65 mg/kg). Both testes were removed. The right epididymis from all rats was removed, weighted and minced with a scalpel blade in the mid-to-distal region of the epididymis. The suspension was kept at 37°C for 5 minutes to allow for the sperm to disperse in the medium. The sperm suspension was gently mixed 20 times and placed in a hemocytometer, and total numbers of the sperms were counted under a Nikon microscope (Nikon Eclipse E600) at a final magnification of ×400 according to the method. Two samples were counted per epididymis and average readings were presented in the final results. The motility of sperm was evaluated directly after mincing in drop of sperm suspension, microscopically. Non-motile sperm numbers were first determined, followed by counting of total sperm. Sperm motility was expressed as percent of motile sperm of the total sperm counted according.

Semen analysis: Sperm morphology
The sperm suspension was stained with Eosin according. The following sperm abnormalities were counted in two separate fields in each of the sperm samples described above: Absence of head, absence of tail, tail bending, tail coiling, mid-piece curving and mid-piece bending.

Results
The results obtained are summarised in Figures 1 and 2.

Sperm count and motility
There were significant gradual decreases in the number and motility of epididymal sperms [Text-Figure 1] in groups of rats exposed to HA during the first 3 weeks of HA exposure (HG1-HG3) with a maximum decreases to seen in HG3. However, the sperm count started to recover gradually on week 4 of HA exposure (HG4) and during all the periods of the reversal protocol achieved by returning rats to LA area (RG1-RG4). The maximum improvement is sperm count and motility was seen in RG3 and RG4 which were not significantly different when compared to each other. The ANOVA test revealed that, in spite of the improvement in sperm count which reach (109.3 ± 6.057 and 113.9 ± 8.967) in RG3 and RG4, their levels remained significantly lower as those obtained in the control LA rats (129.2 ± 11.67).

Sperm morphology
The data showed gradual increase in headless, tailless and coiled tailed sperm abnormalities in HG1, HG2 and HG3 rats, 12.7, 11.6 and 6 folds, respectively; with maximum increases in HG3 (5 folds) in compared to control group. However, partial recovery was seen in HG4 and in all reversal groups. The ANOVA test revealed that individual abnormalities of the heads and tails reported in HG4, RG1 and RG2 were significantly lower than those reported in HG1, HG2 and HG3 [Table 1]. However, complete recovery in all sperms abnormalities were achieved after 3 and 4 weeks of returning hypoxic rats to LA area (RG3 and RG4) [Table 1].

Text-figure 1: Changes in epididimal sperm count (A) and motility (B) levels in the control and all experimental groups of rats. Values are expressed as Mean ± SD for 8 rats in each
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Table 1: Characterization of sperms morphology in the epididymis of rats in the Control and Experimental groups

| Groups | Absence of tail | Absence of head | Tail bending | Tail bending | Tail coiling | Midpiece pending | Total abnormality |
|--------|----------------|----------------|-------------|-------------|-------------|-----------------|------------------|
| Control | 1.9±0.63   | 1.1±0.17   | 1.11±0.31  | 1.4±0.4    | 3.1±0.40   | 1.30±0.35        | 10.15±2.27       |
| HG1    | 15.5±2.4*  | 9.5±1.22*  | 1.11±0.16  | 1.3±0.32   | 17.2±4.9*  | 1.11±0.16        | 46.02±9.38*       |
| HG2    | 17.6±1.9*  | 9.4±1.16*  | 1.13±0.19  | 1.3±0.21   | 19.3±3.5*  | 1.17±0.15        | 49.99±7.19*       |
| HG3    | 24.9±4.2*  | 14.0±1.29* | 1.08±0.15  | 1.2±0.27   | 18.6±2.3*  | 1.13±0.10        | 61.01±8.38*       |
| HG4    | 10.4±1.1*  | 5.7±1.82*  | 1.11±0.16  | 1.3±0.16   | 14.7±3.3*  | 1.22±0.27        | 34.54±7.14*       |
| RG1    | 10.7±1.1*  | 6.2±1.25*  | 1.06±0.13  | 1.1±0.18   | 14.7±3.4*  | 1.23±0.19        | 34.22±4.82*       |
| RG2    | 7.7±1.8*   | 7.4±1.33*  | 1.10±0.16  | 1.0±0.08   | 13.8±1.9*  | 1.16±0.11        | 32.28±5.37*       |
| RG3    | 2.1±0.2*   | 2.8±0.69*  | 1.10±0.29  | 1.1±0.20   | 3.8±0.18*  | 1.23±0.20        | 10.7±2.05*        |
| RG4    | 2.0±0.3*   | 2.0±0.17*  | 1.07±0.14  | 1.2±0.16   | 2.3±0.39*  | 1.16±0.14        | 10.14±1.96*       |

Figure 1: (a, b, c, d, e and f): Photomicrographs of sperm morphology analysis obtained from the control and hypoxic groups. Increased number of sperm abnormal morphologies including absence of head, (arrow head), tail (short arrow) and tail coiling (long arrow) were seen in all hypoxic groups. B: HG1, C: HG 2, D: HG3 and E: HG4. H = Hypoxic, G = Group, R = Reversal

Figure 2: (a, b, c and d): Photomicrographs of sperm morphology analysis obtained from the control and hypoxic groups returned to see levels. Decreased creased number of sperm abnormal morphologies including absence of head, (arrow head), tail (short arrow) and tail coiling (long arrow) were seen photomicrographs A (RG1) and B (RG2) and normal sperm morphology were seen in photomicrographs C (RG3) and D (RG4). H = Hypoxic, G = Group, R = Reversal

Discussion

The present study utilize the effect of natural hypoxia on semen parameters in rats of same species exposed to natural areas of HA and then LA.

A deleterious effect on human and animals reproductive function has been attributed to exposure to HA.\textsuperscript{[9‑11]} have studied the effect of prolonged stay at high altitude (1400 m above sea level) and reported that, high attitude hypoxia causes adverse effects on semen quality and reproductive hormones, and these effects are reversible.

Increased fertility changes are observed in mountaineers, workers, and border personnel in situations that imply acute transfer to highlands.\textsuperscript{[12]} Hypobaric hypoxia (HH) is responsible for altering reproductive function in humans and animals.\textsuperscript{[13‑15]} Furtherly, reducing the number of offspring born at high altitude\textsuperscript{[16]} and mostly related to male’s factor rather than female’s factor.\textsuperscript{[17]}

Table 1: Characterization of sperms morphology in the epididymis of rats in the Control and Experimental groups. Values are expressed as Mean ± SD for 8 rats in each group. Values were considered significantly different at $P < 0.05$. *Significantly different when compared to control group. \textsuperscript{a}Significantly different when compared to HG1. \textsuperscript{b}Significantly different when compared to HG2. \textsuperscript{c}Significantly different when compared to HG3. \textsuperscript{d}Significantly different when compared to HG4. \textsuperscript{e}Significantly different when compared to RG1. \textsuperscript{f}Significantly different when compared to RG2. H = Hypoxic, G = Group, R = Reversal.
The production of mature functional sperm takes place in the seminiferous tubes that release immature spermatooza and the epididymis where the spermatozoa mature and are stored.\(^{[8,19]}\)

In the current study, natural HA exposure resulted in significant decreases in total epididymal sperm count and motility and resulted in increased total sperm abnormality including absence of tail and head and tail coiling. These changes occur during the first 3 weeks of HA exposure with the peak decrease in the third week. Also, significant concomitant decreases in testes and epididymis relative weights were noticed during these periods. These results are in accordance with many published articles on the effect of simulated HH. Studies of acute simulated HA at moderately and extremely HA showed results in damage to all testicular cells, including spermatogenic and somatic elements.\(^{[17‑20]}\) Most of these studies in humans have described low sperm counts and mobility after several weeks of HA exposure.\(^{[18‑21]}\)

Also, similar effects were reported in rodents including highly vacuolated Sertoli cells, decreased germ cell numbers, sperm count and motility, increased abnormal sperm and pyknotic germ cell, expansion of testicular blood vessels and Leydig cell number reduction.\(^{[1‑8]}\) Even more, Morphological studies have revealed that chronic hypoxia causes significant decreases in testicular and epididymal masses.\(^{[13,18]}\) However, our current study showed that partial recovery in the levels of these parameters started to be reversed during the 4\(^{th}\) week of HA exposure. Also, this amelioration in these parameters started gradually to recover after returning to LA area with complete recovery to be seen after 3 weeks of returning the animals to LA area. Supporting to our findings, chronic hypoxia induces a state of reversible oligozoospermia in healthy men.\(^{[14]}\) It has been reported that the mechanism by which HH generate impairment in germ cell development, decreased sperm count and motility.

Our findings are in accordance to many authors who reported similar effect in other tissues.) found that at a simulated HA, decreased levels of SOD occurred in the liver and lungs of the animals and the activity of glutathione peroxidase (GPX) also decreased in their livers. compared the activities of GPX in serum of native highlanders and subjects from sea level and found that decreased levels of SOD occurred in the liver and lungs of the animals. (Similar effect in other tissues.) found that at a simulated HA, decreased levels of SOD occurred in the liver and lungs of the animals and the activity of glutathione peroxidase (GPX) also decreased in their livers. Compared with sea level men, decreased levels of SOD occurred in the liver and lungs of the animals. (Similar effect in other tissues.) found that at a simulated HA, decreased levels of SOD occurred in the liver and lungs of the animals and the activity of glutathione peroxidase (GPX) also decreased in their livers.

On the other hand significant and gradual amelioration in the levels of MDA and activities of both SOD and GPXs in these rats testes homogenates were seen during the 4\(^{th}\) week of HA exposure and during all the period of returning the rats to LA area. Complete recovery of these oxidative stress parameters were seen after the 3\(^{rd}\) and 4\(^{th}\) weeks of LA return.\(^{[22]}\) The recoveries in these parameters are in accordance with the recovery pattern noticed previously in regards to semen analysis and sex organs weights. Together, these findings clearly demonstrates the role of HA generated ROS and oxidative stress in poor reproductive parameters seen in hypoxic rats in this study and the reversibility of these ROS could offer an excellent target for future research using LA therapy or antioxidants.

Conclusion

The present study is an experimental rat model of exposure to natural high altitude (HA) demonstrated that exposure to hypoxia (3150 m) is coupled with adapted reversible changes in semen. Particularly, these included decreased sperm count and motility with a concomitant increase in sperm abnormalities, during the first 3 weeks of HA exposure. On the other hand, it seems that partial recovery start after 4 weeks of HA exposure, and gradually increased after return to LA area (40 m) to reach completely recovery in all parameters at least 3 weeks of return to LA area. To best of our knowledge, this is first studies in literature that demonstrates the time-course of nearly complete recovery in the levels of these reproductive parameters in HA hypoxic rats after return to LA areas.

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Conflicts of interest

There are no conflicts of interest.

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