Chapter 18: Intermittent Hypoxia Remedies Male Sub-fertility, in: Intermittent Hypoxia and Human Disease. Ed. Xi L, Serebrovskaya TV, Springer-Verlag, 2012, pp121-127. ISBN: 978-1-4471-2905-9

R James Swanson[1] and Zoya Serebrovska[2]

[1]Professor of Biological Sciences and University Professor, Biological Sciences Department, Old Dominion University, Norfolk, Virginia, 23529, USA; [2]Assistant Professor, Bogomoletz Institute of Physiology, Kiev 01601, Ukraine

Abstract

Progressive motility with high velocity that exhibits normal lateral head displacement is one of the most important characteristics of spermatozoa directly influencing their fertilizing capacity. A subfertile condition often depends more on sperm motility than count. Application of non-invasive methods of motility stimulation is an important treatment modality for male infertility.

In this chapter, we demonstrate the efficacy of an intermittent hypoxic training (IHT) regimen on improving sperm motility in humans. The IHT consisted of 14 consecutive days having four repetitions of 5-7 min each of induced hypoxia interspersed with 5 min periods of ambient inspiration. Initial inspiration began with atmospheric air (20.9%) O_2. During the procedure, inspired O_2 fell to a value of 5-7% with the final arterial O_2 saturation typically at 84-85%. This IHT regimen produced a stimulatory effect on male reproductive function in the gametes and this significant increase in the number of spermatozoa with high motility was coupled with a decrease of seminal plasma chemiluminescense (ChL) while sperm count and morphology did not change. Motility and free radical status are some of the most labile parameters when compared to other characteristics of male reproductive function, and their response to external influences is very rapid. Sperm count is more inert. Of course, sperm count also depends on spermatozoal survival. Lack of mitochondrial enzyme activity, high energy phosphates, oxidative stress or other stress factors can lead to decreased motility and eventually to apoptosis or necrosis. The increase we have found in motility after 2 weeks of IHT implies that experimenting with the parameters and regimens of the IHT might produce an increase in sperm count along with the motility increase. Our future investigations will include prolonged IHT duration in multiple protocols.

We propose that the mechanism of sperm motility augmentation that occurred in our study is due to inhibition of free radical processes. Positive changes in free radical processes should lead to membrane stabilization and improvement of spermatozoal function. Decrease of free radical levels in blood and increase of erythrocyte superoxide dismutase activity could be two likely causes of decreased free radical oxidation in sperm leading to our improved motility results.

LIST OF ABBREVIATIONS

Term	Definition	Term	Definition
ChL	chemiluminescense	NADP	Nicotinamide adenine dinucleotide phosphate
DNA	deoxyribonucleic acid	pChL	peak ChL
HE	high-energy	ROS	reactive oxygen species
iChL	initiated ChL	SEM	standard error of the mean
IHT	intermittent hypoxic training	sChL	spontaneous ChL
MEA	mitochondrial enzyme activity	SOD	superoxide dismutase

2 Introduction

According to most published data, the influence of a chronic or intermittent hypoxic treatment or experience on spermatogenesis and spermatozoal fertilizing capacity is always negative. Chronic or intermittent hypoxia would generally never be considered as a cure for sterility but quite the reverse;

completely normal males of any species, including humans, become infertile or subfertile after exposure to certain doses of hypoxia [1]. Spanish emigrants who moved to Latin America in the sixteenth century experienced this high altitude problem when they could not conceive for decades [2]. The same problem occurred in domestic animals brought from Europe; they could not produce offspring at altitudes higher than 3,500 to 4,000 meters [3]. Nevertheless, a reduction in oxygen partial pressure has not caused fertility problems for the plateau aborigines or their animals; a privilege achieved by centuries of adaptation [4].

Present day investigations would indicate that no significant changes have taken place in the male response to hypoxia over the last four centuries. Present day investigations on mountaineers [1,2,5] found considerable inhibition of spermatogenesis in long-term sojourns at high altitude, both in permanent and intermittent regimes. In 1968, Donayre, et al., reported[2] a significant decrease in sperm count and motility with marked and sustained increases in abnormal forms in semen samples from 9 volunteers who lived at 14,000 feet (about 4,000 m) for 28 days. The men's levels of testosterone decreased while fructose concentrations rose. Decreases in motility did not parallel the increase in abnormal forms. Similarly there was no clear relationship between decreased motility and increased fructose. The loss of motility without a significant change in the percentage of live spermatozoa could have been due to a toxic, but non-lethal effect of hypoxia on the energy-utilizing mechanism of the spermatozoa or on reduced resources.

Verratti V, et al., reported[5] results of a study on 6 mountaineers who spent 26 days at altitudes of 2000 to 5600 m. Their sperm count was cut in half with a 20% drop in motility and a 20% increase in abnormal or immature forms after this high-altitude exposure. A complete recovery of sperm parameters occurred only after 6 month at sea level. According to the authors, the main mechanism of sperm damage was thought to be an increased generation of reactive oxygen species (ROS). Much reproductive pathology pathogenesis is based on the excess of ROS[6-8]. At an altitude of 4,000 m oxygen is only 63% of sea level air. Tissue hypoxia leads to mitochondrial respiratory chain overloading and ROS accumulation with consequent augmentation of membrane fluidity and DNA damage. This damage, if it occurs during spermatogenesis, before the final nuclear compaction, cannot be repaired in the mature spermatozoon because of the chromatin's 6-fold increase in condensation.

Similar results have been obtained in studies on animals (monkeys and rats)[9-14]. Acclimation to altitudes of 3,500 to 4,000 m leads to decreased sperm count and motility and increased abnormal forms. The main pathogenic ways are: decrease in mitochondrial enzyme activity (MEA) and high-energy (HE) phosphates, ROS accumulation and increased temperature in the testes due to vessel growth.

Saxena DK,[9] observed the following in the rhesus monkey after 21 days at 4,411 m: non-reversible reduction of sperm count and motility, sperm epithelium degeneration, arrest of spermatogenesis and both increased pH and fructose concentration.

Cikutovic M,[10] studied the adaptation of rats to high altitude in an intermittent regime: 7 days at 3,400 followed by 7 days sea level, repeated cyclically 7 times. A significant decrease was found in epididymal spermatozoal concentration, seminiferous tubule diameter, and height of spermatogenic epithelium. These changes were proportional to the hypoxia exposure. In addition to tissue hypoxia, the authors considered the increase of testicular temperature as a possible pathogenic way for the spermatogenic damage seen in these animals.

In a study by Gasco,[11] rats resided at 4,340 m for 42 days. In just seven days a significant decrease in epididymal spermatozoal concentration occurred in the II-III and IX-XII spermatogenic stages.

Farias,[12] studied rats at 4,600 m (PO_2 = 89.6 mmHg) for 60 days. Significant changes in testis morphology, metabolism of round spermatids and death of spermatogenic cells of all stages of spermatogenesis was observed. Similar data were obtained by Shevantaeva and Kosyuga[13] as well as Liao[14].

In all the studies we have cited, humans and animals were exposed to hypoxia of about 4,000 m for weeks. Such dosages clearly negatively influence spermatogenesis and spermatozoal function. Similar phenomenon of decompensation in circulatory and immune systems appear after long-term exposure to altitudes between 4.000 to 5.000 m.[15]

In this chapter, we propose that a mild hypoxic dosing, maintained below the threshold of maladaptation, would produce a stimulatory effect on male reproductive function in the gametes; not inhibitory. Physiological correction within several major body systems has been attained by intermittent hypoxic therapy in a number of different laboratories.[16-18] Therefore we are confident that an effective regimen of training can be experimentally defined for improving all areas of male reproductive function.

Another reason for our optimism is the proven positive influence of intermittent hypoxia on antioxidants.[19-21] Free radical oxidation in controlled amounts is necessary for proper preparation of spermatozoa for the final fertilization phases of capacitation and hyperactivation.[22,23] A special membrane enzyme, NADP oxidase, is responsible for producing this limited superoxide synthesis in spermatozoa. However, excesses of free radicals cause damage of sperm membranes and higher levels of ROS can lead to DNA damage, apoptosis or necrosis, inhibition of spermatogenesis, increases in abnormal forms and decreased motility.[24,25] Free radical damage is especially dangerous for haploid cells because no repair mechanisms for DNA exist at this developmental stage. Moreover, human spermatozoa are very sensitive to free radical damage because of high concentrations of non-saturating acids.[26] The extent of damage depends on the level of oxidative stress, which in turn depends on the balance between pro- and anti-oxidant processes. The latter is a good predictor of fertility.[27]

Intermittent hypoxia stimulates both ROS production and antioxidant synthesis.[28-31] During 5-minute periods of hypoxia, lack of oxygen causes electron-transport-chain overloading with the resultant accumulation of reduced mitochondrial products. When the subject returns to normoxic breathing, oxygen concentration increases rapidly thereby increasing the probability of superoxide formation. An increase in free radicals stimulates antioxidant formation and thus becomes a non-pharmaceutical antioxidant therapy for spermatozoa.

Another reason to presume a positive influence of intermittent hypoxia on spermatogenesis and sperm function is that hypoxic training increases vascular endothelial growth factor (VEGF), which plays an important role in spermatozoon formation and survival. Caires KC et al.[32] have shown that VEGF increases proliferation and survival of spermatozoa in bovine testis. In the testicular Sertoli and Leydig cells, VEGF is produced and carried to the germinal cell receptors. The number of germinal cells significantly increases in culture when VEGF has been added.[33] Hwang GS et al.[34] reports a dose-dependent stimulation of Leydig cell proliferation with testosterone secretion under VEGF influence.

Thus, intermittent hypoxia produced during a training regimen causes antioxidant stimulation and VEGF secretion. These factors will positively influence sperm parameters. In our research we therefore

investigated the influence of hypoxic training on human sperm function and morphology, and on free radical processes in semen and blood.

3 Methods

This work was approved and authorized by the Ethics Committee for Human Experiments at the Bogomoletz Institute of Physiology. All subjects gave their informed consent at the time of enrollment.

3.1 Design of Study

Our study consisted of two sets of experiments: 1) a comparative retrospective investigation of sperm count and motility in two groups of men from environmentally diverse regions of Ukraine and 2) a prospective study to determine the effect of hypoxic training on improving male reproductive parameters.

(1) The L'viv Group (800 km from Chernobyl; n = 37; mean age = 21 ± 1 yr., SEM) was comprised of healthy male volunteers. These males were students attending the L'viv Military Academy. L'viv is considered to be a "radiation clean" region when compared to Chernobyl and Kiev. Investigation of sperm parameters were done one time in the morning after an overnight fast.

(2) The Kiev Group (n = 22; mean age = 25 ± 1 yr., SEM) was also comprised of healthy male volunteers. These males were students attending the Kiev Military Academy. Although Kiev is approximately 90 km from Chernobyl, its residents have been exposed to varying amounts of radiation since the nuclear reactor accident in April 26, 1986. The men in the two groups had no children.

Subjects from the Kiev group underwent a detailed medical checkup before the course of IHT. All blood and semen investigations were carried out 3 times in the Kiev group: two weeks before IHT; one day before IHT; and in the early morning after an overnight fast, 24 h after completing the 14 d IHT program.

3.2 Sperm Morphology and Motility

Sperm analysis included: sample volume, pH, sperm count, motility and morphological characteristics. The volume, pH, sperm count and motility were completed immediately after obtaining the samples. The pH was measured by a RADELKIS meter. For the sperm count, samples were diluted in an isotonic fixative medium (sodium bicarbonate (5g), formaline (5ml) and distilled water (95 ml)) in a ratio of 1:200, and analyzed with a light microscope at 400x. Sperm motility was determined with an ATS-motility Analyzer (J.C.Diffusion International, La Ferte Frensel) at 37°C. A minimum of 100 cells was examined for each sample. The number of *motile spermatozoa* (as a percent of total) and the number of *spermatozoa of high motility*, i.e., forward velocity greater than 50 μm/s (as a percent of total) were assessed for each sample.

Morphological characteristics were evaluated using the WHO criteria.[35] A minimum of 100 spermatozoa were evaluated on each stained slide. Spermatozoa were measured with an eye micrometer if there were any uncertainty in the normal morphological parameters. The number of spermatozoa with normal morphology (as a percent of total) was determined.

3.3 Chemiluminescence

Blood samples for analyses of ChL and antioxidant enzyme activity in serum were obtained from each subject at 3 separate times as outlined in the Design of Study paragraph. Venous blood was drawn from the median antecubital vein in the early morning after an overnight fast.

Levels of ROS were studied by the chemiluminescence measured on an Analyzer HLMZ-1 (Ukraine) with photo-electric multiplier -130 at -11ºC. The lower boundary of sensitivity for the apparatus was 1 mV. For spontaneous ChL (sChL) 2 mL of the fluid to be examined were put in the cuvette and the amount of luminescence generated over a period of 60 seconds at 37ºC was recorded. For initiated ChL (iChL) 0.02 mL of 3% H_2O_2 solution was added to each cell, immediately post-sChL measurement, and this second burst of luminescence generated over the next 60 seconds was measured. The amount of luminescence generated in the first 4 second after initiation, peak ChL (pChL), was also measured and analyzed.

For sperm analysis, three separate fractions were prepared: whole sperm, seminal plasma (dissolved in physiological saline at a ratio of 1:3) and spermatozoal suspension ($12x10^9$ cells/mL). Blood serum was diluted in physiological saline at a ratio of 1:3.

The intensity of sChL depends on the amount of recombined hydroxyl radicals. After initiation of iChL by hydrogen peroxide a recombination growth occurs because of the Fenton reaction, which is catalyzed by free Fe^{3+}. Thus, the iChL reflects the concentration of superoxide and hydroxyl radicals as well as free iron in the sample.

Catalase activity was measured following Koroliuk MA et al.[36] The activity of SOD was measured following Kostiuk VA et al.[37] while the MDA concentration we measured by the method of Gavrilov VB et al.[38]

3.4 Intermittent Hypoxia Protocols

Intermittent hypoxia sessions were conducted on 14 consecutive mornings between 10:00 and 12:00, 2h after a light breakfast. For each of the 14 sessions, the subject was seated and normobaric isocapnic hypoxia was created with an Hypoxotron, a modified closed spirometer which allows measured reduction of P_{O_2} as the subject respires with concomitant CO_2 absorption,[18,39] for four 5-7 min repetitions of induced hypoxia interspersed with 5 min periods of ambient inspiration. Initial inspired gas contained atmospheric (20.9%) O_2. During the procedure, inspired O_2 fell to a value of 5-7% as the subject breathed into the closed system. The final arterial O_2 saturation was typically 84-85%. End-tidal P_{CO_2} was maintained by soda/lime absorption at the initial pre-test value for each subject, typically 38-40 mm Hg, throughout the intermittent hypoxia session. Subjects easily tolerated the hypoxia periods without any untoward effects.

4 Results and Discussion

4.1 Retrospective Comparative Study

A large difference was found between the two geographically separate groups in both sperm count and motility (Table 1) for this comparative study carried out in 1995.

Table 1: Sperm count and motility in the L'viv and Kiev groups prior to hypoxic training

Parameters	L'viv Group	Kiev Group
Sperm count	206.3±19.1*	148.0±26.8
Total count of spermatozoa	405.9±53.9*	324.0±53.8
Sperm motility (%)	63.0±4.2*	47.2±3.7
Total motile x 10^6	255.7±26,4*	152.9±23.2

* p<0.01

Prior to the hypoxic training, the L'viv group's sperm motility was significantly higher than the Kiev group ($p < 0.01$). Values for total motile and vital spermatozoal count had identical distribution with the same significance. The L'viv military men's total motile value was 1.7x higher than Kiev students. How are these differences to be explained? We found no big differences in their mode of life, professional activities, or the use of substances that have a negative influence on spermatozoa (caffeine, alcohol consumption and smoking). Males from L'viv were a little younger and their frequency of sexual activity was lower than males from Kiev. The spermatozoal concentration in the Kiev group was 40% lower than in the L'viv group. The same relative differences were registered for other sperm parameters: percent motility, percent live/dead and number of morphologically normal spermatozoa. The data suggest that not only in one year, but in ten years after the Chernobyl catastrophe (when studies were done) the influence of radiation contamination factors on spermatogenesis were visible. The group from Kiev with the lower sperm count and motility underwent the 14-day hypoxic training protocol.

4.2 Prospective Study

The results from three separate sample collections from the Kiev males are summarized in the following tables: (1) two weeks before IHT, (2) the day before IHT and (3) the day after the 14-day IHT regimen. Data from the first two collections (pre-IHT training) shows no significant differences between any parameters in either sperm or blood samples. (Table 2)

Table 2: Sperm characteristics before and after IHT

Collections	Cnt 10^9 /ml	Tot Cnt 10^9	M %	Tot M 10^9	**High M %**	N %	Tot N 10^9
I	148.0±16.8	324.0±53.8	63.6±3.8	207.0±7.5	47.2±3.7	27.8±2.6	91.0±8.3
II	132.0±14.2	296.0±42.5	65.5±5.7	214.0±9.1	50.1±3.3	30.6±3.1	88.0±9.4
III	140.0±20.2	316.0±58.9	72.5±8.4	230.0±11.5	61.3±4.1	29.5±4.8	92.0±11.4
Significance							
I-II	NS	NS	NS	NS	NS	NS	NS
II-III	NS	NS	NS	NS	p<0.05	NS	NS

Cnt = count; Tot – total; M = motile; N = normal

4.3 IHT, Sperm Motility and Morphology

After the course of IHT a significant increase (22%) in high motility spermatozoa was observed (Table 2). The percent of motile spermatozoa also increased (11%), but without statistical significance. The pH (7.46-7.54), ejaculate volume (2.5 ± 0.2ml), sperm count and morphological characteristics showed no statistically significant change thus no decrease in any parameters occurred due to IHT.

Therefore, two weeks of our IHT training protocol produced no changes in spermatogenesis and we are confident that IHT does not inhibit the final two weeks of spermatogenesis or the normal physiology and anatomy of mature spermatozoa. The observed increase in motility could reflect a metabolic increase due to substrate accumulation or enzymes activation, and/or an improvement in membrane condition. Thus IHT might be useful as a treatment for men with asthenospermia.

4.4 Free Radical Processes in Sperm

The plasma membrane condition is directly related to free radical processes. IHT led to significant decreases in iChL (23%) and pChL (38%) within seminal plasma (Table 3.).

Table 3: ChL of sperm before and after hypoxic training

	Whole sperm			Seminal plasma		
Col	sChL, imp/m	iChL, imp/m	pChL, imp/4s	sChL, imp/m	iChL, imp/m	pChL, imp/4s
I	147.2±3.0	336.9±37.7	4.6±0.4	168.2±2.4	225.0±22.3	4.6±0.4
II	140.2±3.2	352.8±43.4	4.8±0.5	159.1±9.7	234.5±19.4	4.4±0.5
III	137.2±1.4	310.6±28.7	4.0±0.4	141.2±1.1	190.7±18.8	3.1±0.3
Significance						
I-II	NS	NS	NS	NS	NS	NS
II-III	NS	NS	NS	NS	$p<0.05$	$p<0.05$

Col = collections; Imp = impulses; m = minute; s = second

Table 3: ChL of sperm before and after hypoxic training (Continued)

	Whole sperm (repeated)			Suspension of spermatozoon		
Col	sChL, imp/m	iChL, imp/m	pChL, imp/4s	sChL, imp/m	iChL, imp/m	pChL, imp/4s
I	147.2±3.0	336.9±37.7	4.6±0.4	150.2±3.4	175.9±25.7	4.3±0.4
II	140.2±3.2	352.8±43.4	4.8±0.5	146.2±2.6	180.3±29.8	3.7±0.4
III	137.2±1.4	310.6±28.7	4.0±0.4	143.1±7.5	170.5±30.2	4.0±0.5
Significance						
I-II	NS	NS	NS	NS	NS	NS
II-III	NS	NS	NS	NS	NS	NS

Col = collections; Imp = impulses; m = minute; s = second

These changes in ChL parameters are evidence that IHT caused a decrease of hydroxyl radicals in seminal plasma due to either a decreased OH^* production or plasma antioxidant activation. Most antioxidant activity in the ejaculate is concentrated in the seminal plasma, which is rich in antioxidants, including uric acid, α-tocopherol (vitamin E), and ascorbic acid (vitamin C), and it contains high activity levels of superoxide dismutase (SOD) and catalase.[40] In comparison with the seminal plasma, spermatozoa manifest very low antioxidant activity. They contain α-tocopherol, SOD and glutathione peroxidase.[41] The main source of ROS production in sperm is the respiratory chain and membrane NADPH-oxidase. Changes in the ChL of seminal plasma suggest that antioxidant activity was probably stimulated by IHT. This will be verified in future investigations.

4.5 Free Radical Processes and Antioxidants in Blood

Changes in ChL of seminal plasma were accompanied by changes blood free radical processes. After 14 days of IHT, a significant decrease occurred in blood serum iChL (14%), pChL (21%) and MDA concentration (19%) (Table 4).

Table 4: ChL of blood serum and MDA concentration before and after IHT

Collections	iChL, imp/m	pChL, imp/4s	MDA nmol/L
I	714.4±41.5	74.4±5.6	2.74±0.35
II	720.5±35.7	69.5±4.8	2.67±0.24
III	630.7±28.7	57.2±4.3	2.24±0.21

Significance			
I-II	NS	NS	NS
II-III	$p<0.05$	$p<0.05$	$p<0.05$

Imp = impulses; m = minute; s = second; L = liter

The activity of erythrocyte SOD (42%) was significantly increased after the IHT course while the activity of catalase did not change significantly (Table 5).

Table 5: Activity of erythrocyte SOD and catalase before and after hypoxic training

Collections	SOD (act/mL m)	Catalase (mc mol/L bld)
I	250.0±45	217.0±13
II	224.0±38	202.0±12
III	318.0±40	178.0±23
Significance		
I-II	NS	NS
II-III	$p<0.05$	NS

act = activity; m = minute; mc mol = micromole; L = liter; bld = blood

5 Conclusion

Progressive motility with high velocity that exhibits normal lateral head displacement is one of most important characteristics of spermatozoa, which directly influences their fertilizing capacity. Frequently a subfertile condition depends more on sperm motility rather than count. Application of non-invasive method of motility stimulation could become an important treatment modality for male infertility. Many reliable studies have been done, beginning over 30 years ago,[42,43] using motility enhancement chemicals with conflicting results on fertility as decades have gone by. The areas of investigation have included naturopathic or homeopathic substances with questionable experimental protocol. Further investigations are necessary to substantiate the often anecdotal reports. Researchers need to study the mechanisms of motility changes relative to treatment and search for better regimens of individual training which would allow for optimal use of hypoxic stimulation.

In our study sperm count and morphology did not change. Nevertheless, significant increases in the number of spermatozoa with high motility together with a decrease of seminal plasma ChL were observed. These parameters are some of the most labile when compared to other characteristics of male reproductive function, and their response to external influences is very rapid. Sperm count is more inert. The time for one complete cycle of spermatozool formation is about 2½ months, so the results of spermatogenic stimulation, if it occurs, could only be measured in more longitudinal studies. Our future investigations will include prolonged IHT duration. Of course, sperm count also depends on spermatozoal survival. Lack of MEA or HE phosphates, oxidative stress or other stress factors can lead to decreased motility and eventually to apoptosis or necrosis. The increase we have found in motility after 2 weeks of IHT implies that in certain cases and regimens, an increase in sperm count could easily be achieved.

We propose that the mechanism of sperm motility augmentation that occurred in our study is due to inhibition of free radical processes. Positive changes in free radical processes should lead to membrane stabilization and improvement of spermatozoal function. Decrease of free radical levels in blood and increase of erythrocyte SOD activity could be two likely causes of decreased free radical oxidation in sperm leading to our improved motility results.

6 References

1. Okumura A, Fuse H, Kawauchi Y, et al (2003) Changes in male reproductive function after high altitude mountaineering. High Alt Med Biol 4:349-353
2. Donayre J, Guerra-García R, Moncloa F, et al (1968) Endocrine studies at high altitude. IV. Changes in the semen of men. J Reprod Fertil 16:55-58
3. García-Hjarles MA (1989) Sperm count and seminal biochemistry of high altitude inhabitants and patients with chronic altitude sickness. Arch Biol Med Exp (Santiago) 22:61-67 [In Spanish]
4. Yu LK, Gui JH, Feng J, et al (2007) Comparison of sperm parameters between male adults at different altitudes. Zhonghua Nan Ke Xue 13:122-4 [In Chinese]
5. Verratti V, Berardinelli F, Di Giulio C, et al (2008) Evidence that chronic hypoxia causes reversible impairment on male fertility. Asian J Androl 10:602-606
6. Tunc O, Tremellen K (2009) Oxidative DNA damage impairs global sperm DNA methylation in infertile men. J Assist Reprod Genet 26:537-44
7. Ramya T, Misro MM, Sinha D et al (2010) Sperm function and seminal oxidative stress as tools to identify sperm pathologies in infertile men. Fertil Steril 93:297-300
8. Kumar R, Venkatesh S, Kumar M et al (2009) Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligoasthenozoospermic men. Indian J Biochem Biophys 46:172-177
9. Saxena DK (1995) Effect of hypoxia by intermittent altitude exposure on semen characteristics and testicular morphology of male rhesus monkeys. Int J Biometeorol 38:137-140
10. Cikutovic M, Fuentes N, Bustos-Obregón E (2009) Effect of intermittent hypoxia on the reproduction of rats exposed to high altitude in the Chilean Altiplano. High Alt Med Biol 10:357-363
11. Gasco M, Rubio J, Chung A et al (2003) Effect of high altitude exposure on spermatogenesis and epididymal sperm count in male rats. Andrologia 35:368-374
12. Farias JG, Bustos-Obregón E, Orellana R et al (2005) Effects of chronic hypobaric hypoxia on testis histology and round spermatid oxidative metabolism. Andrologia 37:47-52
13. Shevantaeva ON, Kosyuga YI (2006) Effect of acute hypobaric hypoxia on spermatogenesis and lactate concentration in testicular tissue of male albino rats. Bull Exp Biol Med 141:20-22
14. Liao WG, Gao YQ, Cai MC et al (2007) Hypoxia promotes apoptosis of germ cells in rat testes. Zhonghua Nan Ke Xue 13:487-491 [In Chinese]
15. Serebrovskaya TV (2002) Intermittent hypoxia research in the former Soviet Union and the Commonwealth of Independent States: History and review of the concept and selected applications. High Alt Med Biol 3:205-221
16. Xie A, Skatrud JB, Puleo DS et al (2001) Exposure to hypoxia produces long-lasting sympathetic activation in humans. J Appl Physiol 91:1555-1562
17. Ainslie PN, Poulin MJ (2004) Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. J Appl Physiol 97:149-159
18. Serebrovskaya TV, Manukhina EB, Smith ML et al (2008) Intermittent hypoxia: cause of or therapy for systemic hypertension? Exp Biol Med 233:627-650
19. Hui-guo L, Kui L, Yan-ning Z et al (2010) Apocynin attenuate spatial learning deficits and oxidative responses to intermittent hypoxia. Sleep Med 11:205-212

20. Bertuglia S (2008) Intermittent hypoxia modulates nitric oxide-dependent vasodilation and capillary perfusion during ischemia-reperfusion-induced damage. Am J Physiol Heart Circ Physiol 294:H1914-1922
21. Honchar OO, Man'kovs'ka IM (2007) Glutathione system adaptation to acute stress in the heart of rats during different regimes of hypoxia training. Ukr Biokhim Zh 79:79-85 [In Ukrainian]
22. O'Flaherty C, de Lamirande E, Gagnon C (2006) Positive role of reactive oxygen species in mammalian sperm capacitation: triggering and modulation of phosphorylation events. Free Radic Biol Med 41:528-540
23. de Lamirande E, O'Flaherty C (2008) Sperm activation: role of reactive oxygen species and kinases. Biochim Biophys Acta 1784:106-115
24. Baumber J, Ball BA, Gravance CG et al (2000) The effect of reactive oxygen species on equine sperm motility, viability, acrosomal integrity, mitochondrial membrane potential, and membrane lipid peroxidation. J Andrology 21:895-902
25. Aitken RJ, De Iuliis GN, Finnie JM et al (2010) Analysis of the relationships between oxidative stress, DNA damage and sperm vitality in a patient population: development of diagnostic criteria. Hum Reprod 25:2415-2426
26. Kruger TF, Acosta AA, Simmons KF, Swanson RJ et al (1988) Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 49:112-117
27. Sharma RK, Pasqualotto FF, Nelson DR et al (1999) The reactive oxygen species total antioxidant capacity score is a new measure of oxidative stress to predict male infertility. Hum Reprod 14:2801-2807
28. Bailey DM, Taudorf S, Berg RM et al (2009) Increased cerebral output of free radicals during hypoxia: implications for acute mountain sickness? Am J Physiol Regul Integr Comp Physiol 297:R1283-1292
29. Altan M, Atukeren P, Mengi M et al (2009) Influence of intermittent hypobaric exposure on SOD and TBARS levels in trained rats. Chin J Physiol 52:106-112
30. Zarubina IV, Nurmanbetova FN, Shabanov PD (2005) Bemithyl potentiates the antioxidant effect of intermittent hypoxic training. Bull Exp Biol Med 140:190-193
31. Manukhina EB, Downey HF, Mallet RT (2006) Role of nitric oxide in cardiovascular adaptation to intermittent hypoxia. Exp Biol Med 231:343-365
32. Caires KC, de Avila J, McLean DJ (2009) Vascular endothelial growth factor regulates germ cell survival during establishment of spermatogenesis in the bovine testis. Reproduction 138:667-677
33. Hägele S, Behnam B, Borter E et al (2006) TSGA10 prevents nuclear localization of the hypoxia-inducible factor (HIF)-1alpha. FEBS Lett 580:3731-3738
34. Hwang GS, Wang SW, Tseng WM et al (2007) Effect of hypoxia on the release of vascular endothelial growth factor and testosterone in mouse TM3 Leydig cells. Am J Physiol Endocrinol Metab 292:E1763-1769
35. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 3rd ed (1992) Cambridge University Press, Cambridge
36. Koroliuk MA, Ivanova LI, Maĭorova IG et al (1988) A method of determining catalase activity. Lab Delo 1988:16-19 [In Russian]

37. Kostiuk VA, Potapovich AI, Kovaleva ZhV (1990) A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation. Vopr Med Khim 36:88-91 [In Russian]
38. Gavrilov VB, Gavrilova AR, Mazhul' LM (1987) Methods of determining lipid peroxidation products in the serum using a thiobarbituric acid test. Vopr Med Khim 33:118-122 [In Russian]
39. Serebrovska TV, Lopata VA, Roy VV et al (2009) Device for breathing with hypoxic mixtures “Hypoxytron” Patent № 44179, IPC A61M 16/00. Ukraine, 25.09.2009, bulletin 18
40. Baker MA, Aitken RJ (2005) Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. Reprod Biol Endocrinol 29:67
41. Williams AC, Ford WC (2004) Functional significance of the pentose phosphate pathway and glutathione reductase in the antioxidant defenses of human sperm. Biol Reprod 71:1309-1316
42. Mrsny RJ, Waxman L, Meizel S (1979) Taurine maintains and stimulates motility of hamster sperm during capacitation in vitro. J Exper Zool 210:123-128
43. Meizel S, Lui CW, Working PK et al (1980) Taurine and Hypotaurine: their effects on motility, capacitation and the acrosome reaction of hamster sperm in vitro and their presence in sperm and reproductive tract fluids of several mammals. Develop Growth Differ 22:483-494