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The effects of hyperbaric oxygen treatment on vigility of spermatozoids: preliminary report

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The aim of this study was to evaluate the effect of hyperbaric oxygen treatment (HBO) to the vigilance of spermatozoids. The sperm samples were obtained from the patients (n=10) with diagnosed oligospermia. The motility of spermatozoids in tested samples was not lower than 30% but it did not exceed the critical proportion of 50%. Three groups of samples were formed, with respectively 10 samples in each group. All of them were exposed to HBO conditions of 2.5 ATA for 90 minutes. Group I was exposed to HBO conditions without any addition of medium. Group II was added 5 ml of EBSS solution. Tyrode's solution in volume of 5 ml was added to group III. The evaluation of functional characteristics of sperm was carried out 30 minutes after completion of HBO treatment. Statistically significant increased in motor activity was registered in three all groups ($p<0.05$). The biggest increased in vigilance of spermatozoids was found in the samples of the group III ($p<0.01$), exceeding the critical proportion of 50% in all of the samples. The obtained results suggest that the acute exposure of the sperm samples to HBO has favorable impact to functional capacity of spermatozoids in view of their better motility.

Keywords: hyperbaric oxygenation, sperm motility, male infertility

The advancement of reproductive medicine is somewhat slower than it is in other branches of medicine. Conventionally, it has been defined that it is the question of infertility if there is no pregnancy within 12 months of matrimony (or common-law marriage) with regular sexual intercourse and no birth control pills (7, 11).

It is well known that men and women are the cause of infertile marriage in 45% of cases, respectively, and both of them in 10% of cases (9). Spermatogram analysis, in regular laboratory practice includes: sperm volume, sperm viscosity, pH value, normal morphologic features, spermatozoon count, the number of motile spermatozoa and rate of their motility (4, 5, 8). Normal spermatogram implies that over 50% of spermatozoa are motile (1, 11). Management of the existing anatomic malformations, hormonal disorders and associated infectious agents are standard procedures in reproductive medicine related to male sterility (15, 17). There is a great need for better and new approaches to this problem (3, 11).

The rationale for our initiative is the fact that oxygen partial pressure (pPO_2) is not measured either within regular laboratory tests or in specialized institutions (6, 16). It is our opinion that its value is crucial for managing the problem described in our study. If the vigilance and motility of spermatozoa are mitochondria, energy-related, then, under conditions of regular energetic status, normal fructose level, the oxygen deficit may actually be the cause of deficiency or insufficiency of energy necessary for motility of male germ cells. Likewise, it may be the cause of accumulation of anaerobic products and altered intracellular pH value that again may lead to such reduction of motor activity that prevents their motility required for contact and penetration of ovular membrane (14). In literature we have found

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just a few organized investigations and appropriate results on possibilities of HBO in the management of male infertility and that fact has encouraged us to proceed with our research (2, 10, 18, 19).

The aim of this study was to evaluate the effect of oxygen applied in hyperbaric conditions to the vigilance of spermatozoa.

Materials and Methods

Study population consisted of the group of 10 men, age between 28 and 35, with residence in Belgrade, Serbia. All of them were married for more than two years, without offspring. All of the patients have voluntarily come to the Hyperbaric medical Center after they have been diagnosed and unsuccessfully treated from oligospermia in other medical facilities. Absence of anatomical malformations of the reproductive system, normal morphologic features of spermatozoa, hormonal disorders and urinary infections were ratified in all of the patients. From case history we have found that all patients were current smokers (within one month prior to the beginning of our research) and six patients have confirmed alcoholic consumption (within 7 day prior to the beginning of our research). All of the patients have been instructed to maintain the abstinence from ejaculation for 3 days before collecting of the specimen. They have been also instructed that during 7 days prior to collection of the specimens, do not ingest any alcohol, drugs, restrain from smoking cigarettes and try to avoid contact with other potentially toxic substances.

A total of 10 specimens were obtained, one from each patient, by masturbation at the clinical site. Once obtained the specimens were kept at the room temperature and within 30 minutes they were examined in the laboratory. Only samples verified to be free from infectious agents, while sperm volume, pH value, fructose level and total count of spermatozoa were within normal limits, were included in future procedures. The motility of spermatozoa in tested samples was not lower than 30% or it did not exceed the critical proportion of 50% (42.3±6.3).

Each sperm specimen was divided in 3 samples and 1 ml of each was used for our experiment. Three groups of samples were formed, with respectively 10 samples in each group. Soon as the laboratory examinations were over, less than one hour after they were obtained, all three groups of samples were exposed to HBO conditions. Samples were placed in specialized experimental hyperbaric chamber and were exposed to pure oxygen at the pressure of 2.5 absolute atmospheres (ATA) for 90 minutes, with 10 minutes periods of compression and decompression. The only difference among samples was the type of solution used for incubation during the treatment. Group I samples were exposed to HBO conditions without any addition of medium. Each sample of group II was added 5 ml of solution that is generally used for separation of spermatozoa in artificial fertilization EBSS solution (in mM: 116.3 NaCl, 5.3 KCl, 26.1 $NaHCO_3$, 1.8 $CaCl_2$, 1.0 NaH_2PO_4, 0.8 $MgSO_4$, 5.5 D-glucose, and 0.03 phenol red). Tyrode's solution (8 g of NaCl, 0.2 g of KCl, 0.2 g of $CaCl_2$, 0.1 g of $MgCl_2$, 0.05 g of NaH_2PO_4, 1 g of $NaHCO_3$, 1 g of d-glucose, and water to make 1000 ml) in volume of 5 ml was added to samples of group III. Solutions were purchased from Sigma-Aldrich S.r.l. Milan, Italy.

The basic evaluation of functional characteristics of sperm was carried out in time interval of 30 minutes after completion of HBO treatment, in the way it was performed with each sample prior to single HBO treatment.

Written approval to use sperm specimens for our research was obtained from each patient. Our research was approved by the Ethical Committee of the School of Medicine, University of Belgrade (the number of permission EK 5/2008 CHM).

Statistical analyses were performed by the software SPSS 12.0 for Windows, using Student's *t*-test and analysis of variance (ANOVA for related measures). Values of $p<0.05$ were taken as statistically significant.

Results

The presence of oxygen delivered directly under hyperbaric conditions resulted in increased motor activity in all groups (Fig. 1). Statistically significant increase in the vigilance of the spermatozoa was registered in all three group of samples ($p<0.05$). Statistically highly significant increased in the vigilance of spermatozoa ($p<0.01$) was registered in the group III. The vigilance of spermatozoa exceeded the critical proportion of 50% in every sample from the group III. Only 4 samples from the group II have reached the critical proportion of 50%. In group I only one sample had reached the critical proportion of 50%. The changes, developed after the effect of hyperbaric oxygen, were manifested by lowering the pH value (Fig. 2) in all three groups of samples. After the HBO treatment the pH value in all three groups remained in alkali range. Statistically significant decrees in pH value was registered in all of the samples of group I and group II in comparison to the results of the samples from group III ($p<0.05$). Considering the fructose level, statistically significant decrease of concentration was measured in the samples of groups I ($p<0.05$) in comparison to the other two groups (Fig. 3).

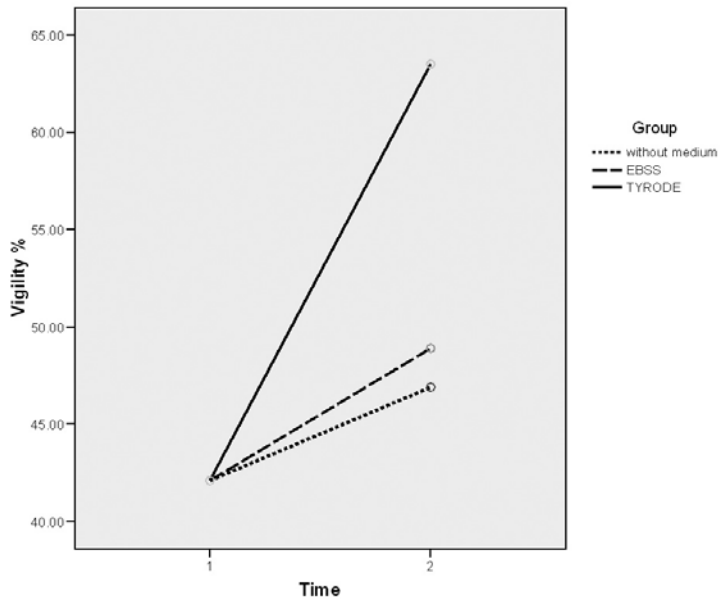

Fig. 1. Motor activity of the spermatozoa
Motility of the samples was evaluated before (Time 1) and after (Time 2) the samples were exposure to pure oxygen at the pressure of 2.5 absolute atmospheres (ATA) for 90 minutes. Group I samples were exposed to HBO conditions without any addition of medium. Each sample of group II was added 5 ml of EBSS solution. Tyrode's solution in volume of 5 ml was added to all samples of group III. Statistically significant increase in the vigilance of the spermatozoa was registered in all three group of samples ($p<0.05$). Statistically highly significant increased in the vigilance of spermatozoa ($p<0.01$) was registered in the group III

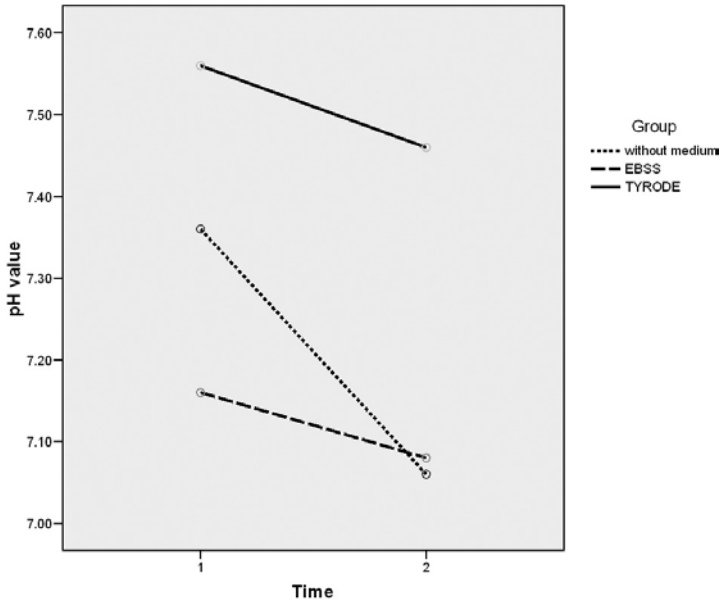

Fig. 2. pH values in the sperm samples
pH value was evaluated before (Time 1) and after (Time 2) the samples were exposure to pure oxygen at the pressure of 2.5 absolute atmospheres (ATA) for 90 minutes. Group I samples were exposed to HBO conditions without any addition of medium. Each sample of group II was added 5 ml of EBSS solution. Tyrode's solution in volume of 5 ml was added to all samples of group III. After the HBO treatment, statistically significant decrease in pH value was registered in samples of groups I and II, in comparison to the values registered in the samples of group III ($p<0.05$)

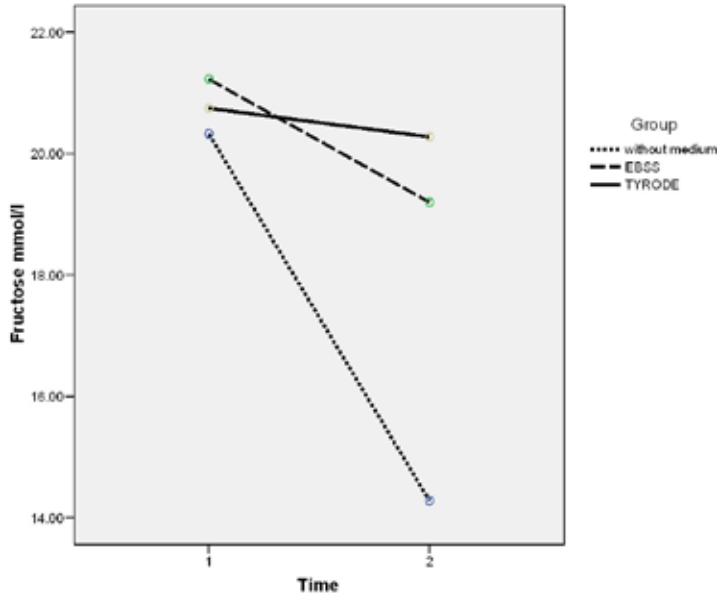

Fig. 3. Fructose level in the sperm samples
Fructose level in the samples was evaluated before (Time 1) and after (Time 2) the samples were exposure to pure oxygen at the pressure of 2.5 absolute atmospheres (ATA) for 90 minutes. Group I samples were exposed to HBO conditions without any addition of medium. Each sample of group II was added 5 ml of EBSS solution. Tyrode's solution in volume of 5 ml was added to all samples of group III. After the HBO treatment, statistically significant decrease in fructose concentration was registered in samples of group I in comparison to the values registered in the other two groups of samples ($p<0.05$)

Discussion

Providing sufficient amount of oxygen to the tissues and cells is crucial for maintaining theirs functional capabilities. As we have previously stated, the vigilance and motility of spermatozoa are mitochondria, energy-related activities. Our presumption was that by exposing the sperm samples to HBO conditions we will insure that, mitochondrial activity within spermatozoa can functions without any disturbance and reach the maximum of its capacities. Increase in motor activity that we have registered confirms that our presumption was correct. Taking that in mind, our opinion and also a suggestion is that oxygen partial pressure (pPO_2) should be measured, when all the other spermatogram findings are within normal range.

Decreasing of the pH values that were registered after the HBO treatment, indicate the accumulation of acid metabolic end products and shows that the metabolic activity of the spermatozoa was increased. It is necessary to point out that in all of the samples pH value remained in alkali range. This is very important to point out because when sperm samples are injected into female reproductive system, during *in vitro* fertilization procedures (IVF), they are placed within environment with lower pH values. Maintaining alkali pH values of sperm samples will help them to preserve their functional capabilities during IVF procedure.

Despite the fact that the biggest decrease in fructose level was registered in sample of group I, which indicates great increase of theirs metabolic activity and significant improvement of theirs vigilance, only one sample from this group has reached critical proportion of 50% motile spermatozoa. Our findings indicate that the effect of HBO treatment is in direct correlation with presence and composition of medium in which these cells were exposed to high pressure of pure oxygen. The results accomplished in the motility of group III samples, and taking into account the composition of added solution, confirm that the issue of vigilance is not isolated and cannot be managed by providing the sufficient quantity of energetic substrates and oxygen only, but there is also series of factors, primarily cell electrolytes, that are to be considered.

Conclusion: The obtained results suggest that the acute exposure of sperm samples to high pressure of pure oxygen has a favorable impact on to functional capacity of spermatozoa in terms of better motility. In addition, the obtained results make this method of involving the patients into HBO treatment program recognized, and, accordingly it might help solving many aspects of the problem of male infertility.

REFERENCES

1. Agarwal A, Sharma RK, Nelson DR: New semen quality scores developed by principal component analysis of semen characteristics. J. Androl. 24, 343–352 (2003)
2. Bar-Sagie D, Mayevsky A, Bartoov B: Effects of hyperbaric oxygenation on spermatozoan motility driven by mitochondrial respiration. J. Appl. Physiol. 50, 531–537 (1981)
3. Balasch J: Investigation of the infertile couple: investigation of the infertile couple in the era of assisted reproductive technology: a time for reappraisal. Hum. Reprod. 15, 2251–2257 (2000)
4. Bonde JP, Ernst E, Jensen TK, Hjollund NH et al.: Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 352, 1172–1177 (1998)
5. Chen X, Zhang W, Luo Y, Long X, Sun X: Predictive value of semen parameters in vitro fertilization pregnancy outcome. Andrologia 41, 111–117 (2009)
6. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST et al.: Sperm morphology, motility, and concentration in fertile and infertile men. N. Engl. J. Med. 345, 1388–1393 (2001)

7. Guttmacher AF: Factors affecting normal expectancy of conception. J. Am. Med. Assoc. 161, 855–860 (1956)
8. Henry JB (2001): Clinical diagnosis and management by laboratory methods. 20th ed. Cambridge University Press, Cambridge
9. Jose-Miller AB, Boyden JW, Frey KA: Infertility. Am. Fam. Physician 75, 894–856 (2007)
10. Kolski JM, Mazolewski PJ, Stephenson LL, Texter J, Grigoriev VE, Zamboni WA: Effect of hyperbaric oxygen therapy on testicular ischemia-reperfusion injury. J. Urol. 160, 601–604 (1998)
11. Lee VM, Wong JS, Loh SK, Leong NK: Sperm motility in the semen analysis affects the outcome of superovulation intrauterine insemination in the treatment of infertile Asian couples with male factor infertility. BJOG. 109, 115–120 (2002)
12. Makar RS, Toth TL: The evaluation of infertility. Am. J. Clin. Pathol. 117, S95–103 (2002)
13. Ombelet W, Cooke I, Dyer S, Serour G, Devroey P: Infertility and the provision of infertility medical services in developing countries. Hum. Reprod. Update 14, 605–621 (2008)
14. Patel S, Panda S, Nanda R, Mangaraj M, Mohapatra PC: Influence of oxidants and anti-oxidants on semen parameters in infertile males. J. Indian Med. Assoc. 107, 78–80 (2009)
15. Rodriguez Peña M, Alescio L, Russell A, Lourenco da Cunha J, Alzu G, Bardoneschi E: Predictors of improved seminal parameters and fertility after varicocele repair in young adults. Andrologia 4, 1277–1281 (2009)
16. Said L, Galeraud-Denis I, Carreau S, Saâd A: Relationship between semen quality and seminal plasma components: alpha-glucosidase, fructose and citrate in infertile men compared with a normospermic population of Tunisian men. Andrologia 41, 150–156 (2009)
17. Uhler ML, Zinaman MJ, Brown CC, Clegg ED: Relationship between sperm characteristics and hormonal parameters in normal couples. Fertil. Steril. 79, 1535–1542 (2003)
18. Van Den Brenk HA, Jamieson D: Hyperbaric oxygen and testicular damage and fertility. Experientia 23, 302–303 (1967)
19. Zadoev SA, Evdokimov VV, Rumiantsev VB, Osmolovskiĭ EO: Hyperbaric oxygenation in the treatment of patients with chronic congestive prostatitis and lower fertility. Urologia 1, 27–30 (2001)