ZERO MAGNETIC FIELD INFLUENCE ON *IN VITRO* HUMAN SPERMATOZOA CELLS BEHAVIOR

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Abstarct The effect of zero magnetic field environments (ZMF) on human semen was investigated in connection with the main natural fertilization parameters: viability and motility of spermatozoa cells. Semen samples were exposed for 30 hours in ZMF created in a pair of Helmoltz coils and in geomagnetic field (GMF) as control at 20°C. Cells population aging was estimated by the decrease of rapid progressive cells viability. An imaging analysis method was used to characterize cells velocity. We found that in ZMF the decrease of cells viability was delayed with about five hours compared to GMF. Rapid progressive spermatozoa cells velocity naturally slows down at 20^0C after four hours; cells become slow progressive or immobile after 20 hours. A significantly enhancement of cells motility was recorded in the first period of ZMF exposure. In this conditions cells velocity is maintained around the initial values even after 20 hours. We concluded that zero magnetic field slow down the aging of spermatozoa cells population, and stimulate cells velocity in vitro conditions.

Key words: zero magnetic fields, geomagnetic field, geomagnetic activity, spermatozoa, semen, spermatozoa cells viability, spermatozoa cells velocity, rapid progressive spermatozoa.

INTRODUCTION

In the previous zero magnetic field (ZMF) investigations we emphasize an unfavorable influence of ZMF conditions on the enzymatic and ion pumps activity of red cells [1–2, 5]. ZMF also significantly increased the aging of erythrocytes, due to reduction of Na^+-K^+ATPase and Ca^{2+}-ATPase activity, and increased hemolysis [5]. The influence of hypomagnetic field on ciliate apparatus of ependymal cells in newborn rats in vivo causes an inhibitory effect, up to absolute stoppage [6]. In this paper we report for the first time the behavior of spermatozoa in ZMF conditions.

The spermatozoa (male germ cells) are haploid cells which carry half genetic material, a 23 single set of chromosomes and have their own mean of motility. Analysis of semen (ejaculated fluid which contains spermatozoa cells) can be done conventionally or using computerized methods to gather information about viability, motility, tail beat cross frequency, straightness of trajectories, form, and

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lateral head displacement. However the main function of these male germ cells in the natural fertilization process is implicitly related to cells viability, and motility [4]. The scope of our investigations was to determine the influence of ZMF medium on male germ cells viability and on cells velocity, respectively on the aging process.

MATERIALS AND METODS

Samples of human semen was incubated 30 hours at room temperature, in natural geomagnetic field (GMF) and in ZMF created in the space delimited by a pair of Helmholtz coils. In this space the static component of GMF was compensated but natural magnetic fluctuations remain operative.

The viability and velocity of rapid progressive spermatozoa cells was determined at three hour intervals in the samples exposed both in GMF and ZMF. The percent of cells viability was estimated microscopically by counting the rapid, slow and immobile spermatozoa using a Bruker-Turk counting chamber. ZMF effect on rapid progressive spermatozoa viability is determined by normalized difference of cells percent recorded in ZMF and GMF as control.

In order to determine the spermatozoa cells velocity we used a sample imaging analysis method to analyze cells trajectories. A sequential shouting of 10 images/set of sample population was recorded on a PC with 1 s^{-1} frequency, using a CCD camera connected to a research microscope. The spermatozoa velocity was estimated at each preselected incubation time by the mean of 6–10 cells path trajectories length/sec. The results were statistical evaluated on 15–30 cells trajectories/10 sec. ZMF effect on rapid progressive cells velocity was estimated as in the spermatozoa viability experiment.

ZMF influence on viability and velocity of rapid progressive spermatozoa is discussed in relation of geomagnetic activity in the period of cells exposure. Geomagnetic activity (GMA) is a measure of the natural geomagnetic field fluctuation and is quantified by *Ap* indices of GMA. These indices were downloaded from National Geophysical Data Center, USA [3].

RESULTS AND DISCUSSION

Fig. 1 and Fig. 2 present the viability and velocity of spermatozoa cells exposed in ZMF and in GMF.

The main capacity of fertilization of a spermatozoa population is attributed to cells with more than 16 μm/s velocity, called rapid progressive spermatozoa. We present here the results obtained on this group of cells. At 20°C, the natural

decrease of rapid cells viability followed a linear slope with a rate of –2.433 ± 0.308%/hour. As we can see in Fig. 1, this process was significantly delayed in ZMF conditions. The initial viability of rapid cells was maintained about four–five hours while in GMF it decreases by 15% after this time interval. Also, the process of cells aging follows the same slope as in GMF but shifted to higher values with the initial difference. Rapid progressive germ cells become slow progressive or immobile after 15 hours (< 5%) in GMF but only after 20 hours in ZMF.

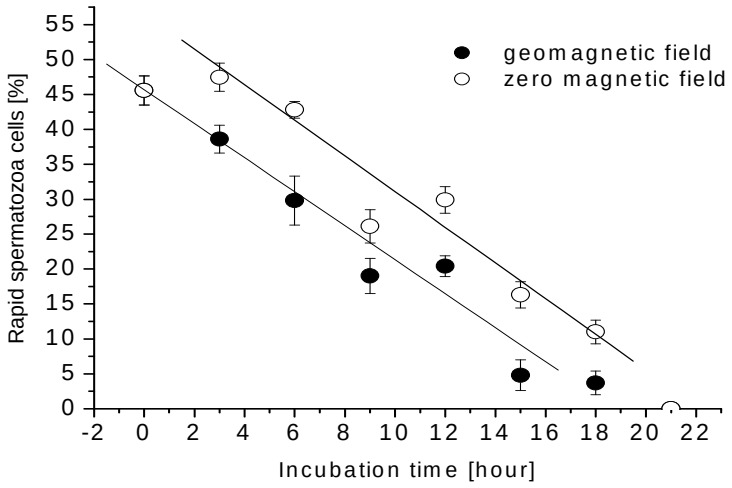

Fig. 1. Viability of rapid progressive spermatozoa in ZMF and GMF as control at different incubation times. Temperature of medium 20 °C.

Spermatozoa cells motility is significantly stimulated in ZMF conditions as we can see in Fig. 2. Even more, in the first period of ZMF exposure the path velocity exceeds the initial value. The normal average rapid progressive spermatozoa velocity varies around 25 μm/s. These results show that in our case, cells velocity fluctuate around the initial value (23 μm/s) roughly for 6 hours in GMF. After 18 hours we found viable male germ cells with 17 μm/s velocity that are still rapid progressive.

In ZMF conditions we recorded a significant stimulation of cells velocity after three hours of exposure. The average of the path velocity was 29 μm/s which is 25% more than the initial value. This value decrease and attained the natural cells motility only after nine hours. The average of cells velocity was close to the initial value even after 18 hours. This suggests that the cells velocity did not decrease significantly in ZMF conditions.

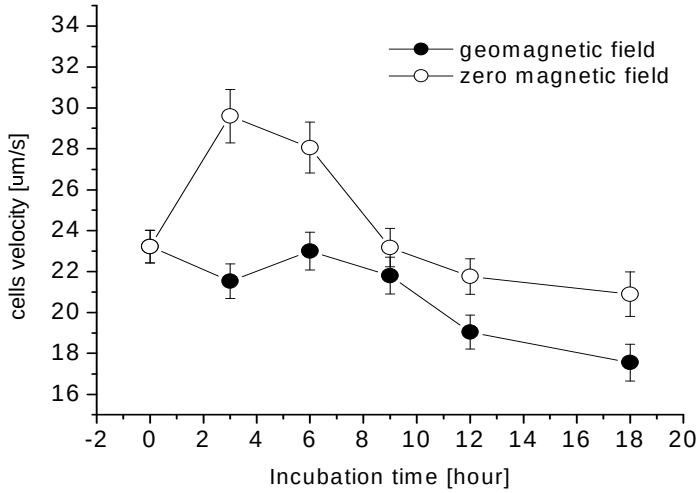

Fig. 2. Rapid progressive spermatozoa velocity in ZMF and GMF as control at different incubation times. Temperature of medium – 20 °C.

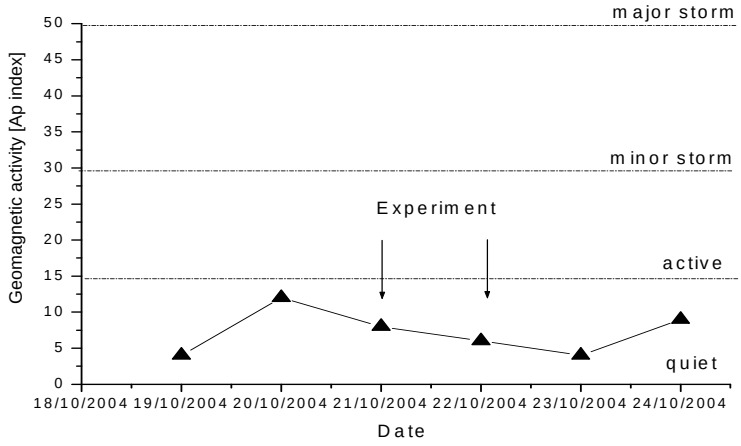

Fig. 2. Geomagnetic activity in the period of spermatozoa cells exposed in ZMF and GMF.

The results presented above emphasize a paradox regarding the dynamics of rapid progressive male germ cells in ZMF conditions. It is possible that the increase of cells velocity was due to the stimulation of some of the slow progressive germ cells which shifts into the rapid progressive cells group.

The experiments were performed during quiet geomagnetic field activity period as we can see in Fig. 3. The interval of the experiment is delimited in the plot by arrows. This indicates that our experimental conditions were not influenced by natural variation of GMA (induced by magnetic storms). We concluded that the effect recorded in ZMF conditions is due to the absence of static geomagnetic field.

Cells motility is driven by the mechanism of molecular motor of motile cells flagella. In comparison with the response of motile bacteria whose motility is inhibited in ZMF, our results show a different behavior in the case of progressive spermatozoa.

CONCLUSIONS

ZMF enhance the viability and increase the velocity of spermatozoa, which is opposite to the behavior of motile bacteria and other similar cells. Also the aging process is delayed in these magnetic field conditions. We suggest that the influence of ZMF is related to the different kind of molecular motor implied in the motility of these cells.

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