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Zero magnetic field influence on human spermatozoa glucose consumption

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Abstract

Free energy released from the hydrolysis of adenosine triphosphate is required for spermatozoa movement, and the spermatozoa velocity is influenced by magnetic field. Since glucose is necessary for maintenance of optimal adenosine triphosphate levels, a question arises: is the human spermatozoa glucose consumption influenced by zero magnetic field conditions? The effect of zero magnetic field on human semen glucose consumption was investigated in connection with the main natural fertilization parameters: viability and motility of spermatozoa cells. We report, for the first time, that human spermatozoa glucose consumption is accelerated in vitro in zero magnetic field conditions.

Keywords: zero magnetic fields, geomagnetic activity, semen, normozoospermia, rapid progressive spermatozoa.

Introduction

Several studies strongly suggest the importance of glucose (Glu) in achieving fertilization (BARAK & al. [1], MAHAVEN & al. [2]). However the main function of these male germ cells in the natural fertilization process is implicitly related to cells viability, and motility (KNUTH & al. [3]).

Mature mammalian spermatozoa have a highly specialized metabolism, their adenosine triphosphate (ATP) demand is only finalized to their motility (MUKAI & al. [4]). It is known that free energy released from the hydrolysis of ATP is required for sperm movement (MUKAI & al. [4]).

Reports shows that a glycolyzable sugar is necessary for hyperactivation of human sperm and maintenance of optimal ATP levels (WILLIAMS & al. [5]). Glu, fructose, or mannose is the only sugars glycolyzable by human sperm. However, no significant concentration of mannose is present in human sperm (D. H. OWEN & al. [6]). Spermatozoa movement is using ATP as major biofuel. It is known that the human spermatozoon relies on glycolysis as the primary ATP source and the main glycolysis bioenergetical substrate is Glu (BARAK & al. [1], MAHAVEN & al. [2], MIKI & al. [7]).

Reports show that in zero magnetic field (ZMF) the cells viability decrease is delayed with about five hours (TRUTA & al. [8]), compared to Earth geomagnetic field (GMF). Also, a significantly enhancement of cells motility is reported in ZMF in vitro conditions (TRUTA & al. [8]). The proportion of rapid progressive spermatozoa, as a result to ZMF exposure, increases (TRUTA & al. [9]). Evidence is provided that, higher than GMF, magnetic field (MF) exposure may have an adverse effect on sperm quality (DE-KUN & al. [10]). Also, magnetic-activated cell sorting for sperm preparation can be done with significant results

(LEE & al. [11]). Reports show that the geomagnetic field has a significant effect on biological orientation, in part on human cells (BINHI [12]).

All cited studies shows, that human spermatozoa are sensitive to magnetic environmental changes, at Earth's geomagnetic field scale level. The objective of this study was to elucidate the effect of ZMF on human spermatozoa glucose metabolic consumption, in vitro, relative to natural GMF condition, at 22^0C ($\pm 0.3^0$C) room temperature.

Experimental

Human semen samples were exposed for 30 hours in ZMF, and in geomagnetic field (GMF) as control. To compensate the GMF we used a pair of Helmoltz coils with an adjustable DC voltage supply. The device was oriented, and the best homogenous exposing place was chosen, after a spatial magnetic map was drawn. Possible electromagnetic fields generators were removed from vicinity. GMF was compensated until the magnetic tester showed less than 150 nT (about 0.5% of the initial value for the horizontal static magnetic field at our research location) but natural magnetic fluctuations remain operative. We consider as initial the measurements that were taken as soon as the semen was liquefied (10- 15 min. after ejaculation). We consider as final the measurements that were taken after 72 hours of exposure.

For the second set of measurements rapid progressive, slow progressive and immobile spermatozoa (RP, SP, I %) were counted using an inversed research microscope, and a Makler counting chamber ($1:10^6$ counting scale) (MAKLER [13], WHO [14]).

Samples were obtained from a group of 30 healthy white male donors, between the ages of 25 and 39, and with semen samples known to be with normozoospermia. Each donor donated one semen sample. Samples collection was always done on the IVF Laboratory premises by ejaculation. The counting was done by 2 researchers on 10 squares, in 5 or 6 different locations of each and every semen sample, and the results was normalized and mediated for each counting measurement set at a time point. Cells population aging was estimated by the decrease of RP cells viability. We used the length of time since ejaculate as the main variable, and we divided the experiment time in 3 or 4 hours time intervals.

A computer assisted semen analysis (CASA) method was used to characterize cells velocity. We used a secondary system composed of a CCD camera, connected to our research microscope, and a PC with video imaging, for counting and velocity measurements control.

Straight path velocity (VAP) was determined for all spermatozoa, visualized in 10 images/set at a time (15-30 cells), using a sequential shouting with 1 s^{-1} frequency. For each velocity measurement set the measurement errors was calculated, and the average result was presented.

Glu concentration in semen was assessed using Glu Ulta-Violet Peak (**GUVP) method** (TRUTA & al. [15]). We use equation (1) for each set of five UV-VIS absorption measurements at a time point, for each and every semen sample analyzed (TRUTA & al. [15]).

$$x = 174.7915\ y \qquad (1)$$

The results are significant at $p<0.05$ level, and the results for the total Glu concentration in human semen x, are in the usual units of mg/100mL. In (1) x represents the value of Glu semen sample concentration, and y is the UV-VIS absorbance value, at 267 nm for the same sample.

For data correlation we used the Correlation method and Fast Fourier Transform (FFT) correlation (Origin 6.1). To measure the correlation between two columns of data, we obtained the resultant lag or index variables, and the correlation result (Origin 6.1). FFT was applied coupling on the index variables, and the correlation result columns. The absolute value of the correlation result (amplitude) will be large when the leftmost dataset is exactly shifted to the right or to the left of the second dataset by the lag value.

Makler undiluted and Makler 1:1 count was tested systematically for absolute agreement using Intraclass Correlation Coefficients (ICC) and the 2-way mixed effects model. Coefficients of Variation (CV) were also calculated, to determine the spread of the repeat counts relative to the mean for each method. The 95% confidence intervals (CI) were also measured for each method. Glu concentration results presented are significant at the $p<0.05$ level.

ZMF influence on spermatozoa Glu consumption is discussed in relation of geomagnetic activity in the period of cells exposure. Geomagnetic activity (GMA) is a measure of the natural GMF fluctuation and is quantified by Ap indices of GMA. These indices were downloaded from National Geophysical Data Center, USA [16].

Results and discussion

VAP for a (52, 9, 39%) semen sample, is decreasing in time in GMF, while for the ZMF exposed specimen, VAP increases for the first 5 hours (Fig.1a).

Same initial behavior is presented for a (57, 11, 32%) semen sample (fig.1b). Average vap is maintained greater in zmf than in gmf, for the entire motility time (fig.1b). The natural vap decrease in time is shifted with about 3.5 hours. After 30 hours of exposure zmf exposed specimen had an average vap of 19.1μm/s (±0.86μm/s) while gmf exposed specimen had an average vap of 16.3μm/s (±1.23μm/s) (fig.1b). Similar behaviors were recorded for all 30 semen samples considered (basic graphs results not presented).

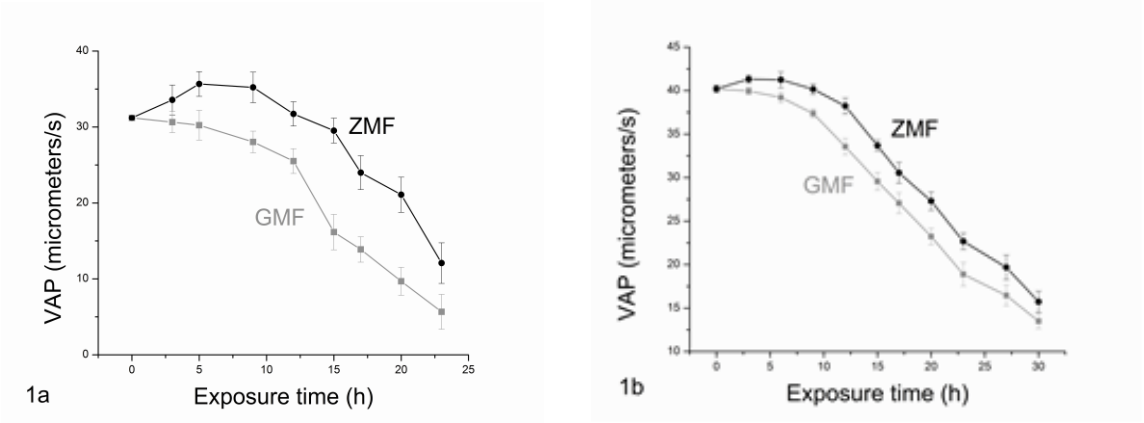

Fig.1 Spermatozoa VAP mean values, after GMF (gray) and ZMF (black) exposure, for a (52, 9, 39%) (1a) and a (57, 11, 32%) (1b) semen samples.

In all others normozoospermic donor specimens, after 22 hours incubation the rapid progressive cells were practically inexistent in GMF and less than 4 % in ZMF (basic graphs not presented). Even after 48 hours some semen samples still contain few SP cells. This is the reason why we choose the total exposure time 72 hours.

Initial VAP, the main motility parameters, and RP after 5h of exposure, were recorded as references, for all samples analyzed (Tab.1).

Tab.1 Initial VAP (columns 3, 8) determined for all semen samples (#). Cell count shown for each sample (col. 2, 7), and RP after 5h exposure in ZMF (col. 4, 9), respective GMF (col.5, 10).

#	INITIAL RP, SP, I (%)	INITIAL VAP (μm/s)	5h RP ZMF (%)	5h RP GMF (%)	#	INITIAL RP, SP, I (%)	INITIAL VAP (μm/s)	5h RP ZMF (%)	5h RP GMF (%)
1	52, 9, 39	31.2±0.36	59	50	16	69, 6, 25	31.7±0.43	77	68
2	57, 11, 32	40.2±0.44	64	54	17	67, 16, 17	39.3±0.37	75	66
3	59, 8, 33	39.5±0.39	66	58	18	63, 9, 28	27.5±0.35	66	60
4	70, 5, 25	37.9±0.46	76	68	19	54, 14, 32	41.2±0.46	64	52
5	66, 10, 24	27.7±0.47	71	63	20	51, 17, 32	36.3±0.44	58	59
6	71, 0, 29	25.9±0.36	79	68	21	69, 7, 24	29.6±0.38	74	66
7	60, 11, 29	31.6±0.38	67	59	22	62, 13, 25	31.8±0.43	68	60
8	53, 14, 33	37.3±0.43	57	51	23	51, 15, 34	36.1±0.44	57	50
9	57, 17, 26	31.6±0.40	62	54	24	72, 13, 15	47.9±0.38	76	67
10	56, 8, 36	27.1±0.35	63	54	25	60, 7, 33	36.3±0.33	65	57
11	57, 24, 19	27.9±0.42	61	55	26	61, 9, 30	29.7±0.29	66	57
12	50, 24, 26	41.1±0.45	54	48	27	54, 6, 40	37.4±0.41	58	52
13	59, 7, 34	33.3±0.34	67	57	28	59, 8, 33	25.2±0.36	68	56
14	58, 12, 30	29.5±0.32	65	55	29	63, 14, 23	36.7±0.45	67	60
15	64, 15, 21	26.7±0.36	70	62	30	64, 6, 30	37.7±0.42	73	62

Tab.2 Initial GUVP Abs (columns 2, 6) determined for all semen samples (#). Final GUVP Abs determined for all samples (#) after GMF (columns 3, 7), and ZMF (columns 4, 8) exposure.

#	Glu peak GMF initial (Abs)	Glu peak GMF final (Abs)	Glu peak ZMF final (Abs)	#	Glu peak GMF initial (Abs)	Glu peak GMF final (Abs)	Glu peak ZMF final (Abs)
1	0.2596±0.0125	0.1493±0.0096	0.0715±0.0075	16	0.2728±0.0136	0.1384±0.0108	0.0357±0.0045
2	0.2396±0.0126	0.1540±0.0099	0.0591±0.0067	17	0.2928±0.0126	0.1509±0.0119	0.0466±0.0046
3	0.2877±0.0100	0.1727±0.0111	0.0824±0.0096	18	0.2631±0.0143	0.1742±0.0112	0.0840±0.0089
4	0.3083±0.0140	0.2380±0.0153	0.1229±0.0114	19	0.2528±0.0116	0.1571±0.0101	0.0762±0.0085
5	0.2791±0.0134	0.2178±0.0140	0.1104±0.0137	20	0.2396±0.0136	0.1913±0.0123	0.1073±0.0112
6	0.3214±0.0142	0.2225±0.0143	0.1104±0.0147	21	0.3003±0.0132	0.1696±0.0109	0.0591±0.0078
7	0.2379±0.0136	0.1447±0.0093	0.0466±0.0056	22	0.2694±0.0103	0.1649±0.0106	0.0793±0.0094
8	0.2602±0.0121	0.1649±0.0106	0.0778±0.0052	23	0.2265±0.0111	0.1384±0.0108	0.0529±0.0062
9	0.2791±0.0123	0.2053±0.0132	0.1151±0.0136	24	0.3283±0.0142	0.2318±0.0149	0.1182±0.0112
10	0.2717±0.0132	0.2038±0.0131	0.1136±0.0126	25	0.2533±0.0114	0.2007±0.0129	0.1058±0.0132
11	0.2494±0.0116	0.1587±0.0102	0.0715±0.0083	26	0.2591±0.0123	0.1524±0.0098	0.0466±0.0052
12	0.2425±0.0136	0.1867±0.0120	0.1058±0.0125	27	0.2671±0.0116	0.2085±0.0134	0.1182±0.0142
13	0.2957±0.0114	0.2053±0.0132	0.1151±0.0142	28	0.2379±0.0121	0.1509±0.0109	0.0575±0.0085
14	0.2820±0.0145	0.1649±0.0106	0.1167±0.0122	29	0.2774±0.0132	0.1898±0.0122	0.0933±0.0097
15	0.2722±0.0125	0.1493±0.0096	0.0638±0.0075	30	0.2722±0.0129	0.1664±0.0107	0.0637±0.0087

GUVP Abs (± SD, standard error of deviation) resulted from measurements for all 30 semen samples are presented (Tab.2).

Glu concentration resulting calculated, using equation (1), from all semen samples are presented (Tab.3).

Our results suggest that there is a correlation between the initial Glu concentration in nomozoospermic human semen, and sperm RP motility parameter. Normozoospermic human semen with greater Glu concentration, have greater RP cells proportion (Tab.1, Tab.3).

Tab.3 Initial Glu concentration (columns 2, 6) for all semen samples (#). Final Glu concentration, determined after GMF (columns 3, 7), and ZMF (columns 4, 8) exposure.

#	Glu concentration initial GMF (mg/100mL)	Glu concentration final GMF (mg/100mL)	Glu concentration final ZMF (mg/100mL)	#	Glu concentration initial GMF (mg/100mL)	Glu concentration final GMF (mg/100mL)	Glu concentration final ZMF (mg/100mL)
1	45.3±2.4	26.1±2.8	12.5±2	16	47.7±2.8	24.2±2.2	6.2±1.7
2	41.8±2.5	26.9±2.1	10.3±1.8	17	51.2±2.5	26.3±2.3	8.1±1.7
3	50.2±2	30.1±2.3	14.4±2.1	18	46±2.8	30.4±2.3	14.6±2.1
4	53.9±2.7	41.6±2.8	21.4±2.3	19	44.2±2.3	27.4±2.2	13.3±2.1
5	48.8±2.7	38±2.6	19.3±2.6	20	41.9±2.6	33.4±2.5	18.7±2.3
6	56.2±2.7	38.8±2.7	19.3±2.7	21	52.5±2.6	29.6±2.2	10.3±2
7	41.6±2.7	25.2±2.1	8.15±1.8	22	47.1±2.1	28.8±2.2	13.8±2.1
8	45.5±2.4	28.8±2.2	13.5±1.8	23	39.6±2.2	24.2±2.2	9.2±1.8
9	48.8±2.4	35.9±2.5	20.1±2.6	24	57.3±2.7	40.5±2.7	20.6±2.3
10	47.5±2.7	35.6±2.6	19.8±2.5	25	44.2±2.3	35±2.6	18.4±2.6
11	43.6±2.3	27.7±2.2	12.5±2.1	26	45.2±2.4	26.6±2.1	8.1±1.8
12	42.4±2.7	32.6±2.5	18.4±2.4	27	46.6±2.3	36.4±2.6	20.6±2.7
13	51.7±2.3	35.9±2.6	20.1±2.7	28	41.5±2.4	26.3±2.2	10.0±2.1
14	49.3±2.8	28.8±2.2	20.3±2.5	29	48.5±2.6	33.1±2.5	16.3±2.1
15	47.6±2.4	26.1±2.1	11.1±2	30	47.5±2.5	29.1±2.2	11.1±2.1

To analyze this correlation we used FFT correlation, as described above. The results are presented (Fig. 2a, Fig. 2b).

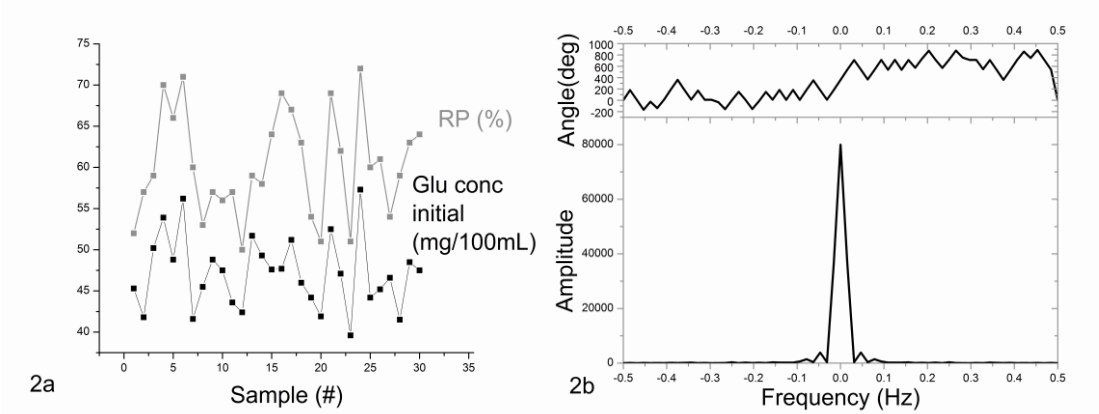

Fig.2 Initial RP cells proportion (gray), and Glu concentration (black) correlation, for all semen samples (2a). FFT shows a significant absolute value (amplitude) of the correlation result (2b).

From (Tab. 3) average glucose concentration in human semen can be calculated initial, and after 72 hours of exposure in GMF, respective ZMF:
Glu concentration initial = 41.17±4.38 mg/100mL
Glu concentration final GMF = 31.02±5.04 mg/100mL
Glu concentration final ZMF = 14.73±4.74 mg/100mL
From (Tab.3) total Glu consumption (72 hours) for each semen sample can be calculated (Tab.4).

Tab.4 Semen samples (#) Glu consumption, after 72 hours exposure in GMF (columns 3, 7), respective ZMF (columns 4, 8).

#	Initial RP (%)	Glu consumption GMF (mg/100mL)	Glu consumption ZMF (mg/100mL)	#	Initial RP (%)	Glu consumption GMF (mg/100mL)	Glu consumption ZMF (mg/100mL)
1	52	19.1±5.2	32.7±4.4	16	69	23.4±5	41.4±4.5
2	57	14.8±4.6	31.4±4.3	17	67	24.8±4.8	43±4.2
3	59	20±4.3	35.7±4.1	18	63	15.5±5.1	31.3±4.9
4	70	12.2±5.5	32.4±5	19	54	16.7±4.5	30.8±4.4
5	66	10.7±5.3	29.4±5.3	20	51	8.4±5.1	23.1±4.9
6	71	17.3±5.4	36.8±5.4	21	69	22.8±4.8	42.1±4.6
7	60	16.3±4.8	33.4±4.5	22	62	18.2±4.3	33.2±4.2
8	53	16.6±4.6	31.9±4.2	23	51	15.3±4.4	30.3±4
9	57	12.8±4.9	28.6±5	24	72	16.7±5.4	36.6±5
10	56	11.8±5.3	27.6±5.2	25	60	16.7±4.9	25.7±4.9
11	57	15.8±4.5	31±4.4	26	61	18.5±4.5	37±4.2
12	50	9.7±5.2	23.9±5.1	27	54	10.1±4.9	25.9±5
13	59	15.7±4.9	31.5±5	28	59	15.1±4.6	31.4±4.5
14	58	20.4±5	28.9±5.3	29	63	15.3±5.1	32.1±4.7
15	64	21.4±4.5	36.4±4.4	30	64	18.3±4.7	36.3±4.6

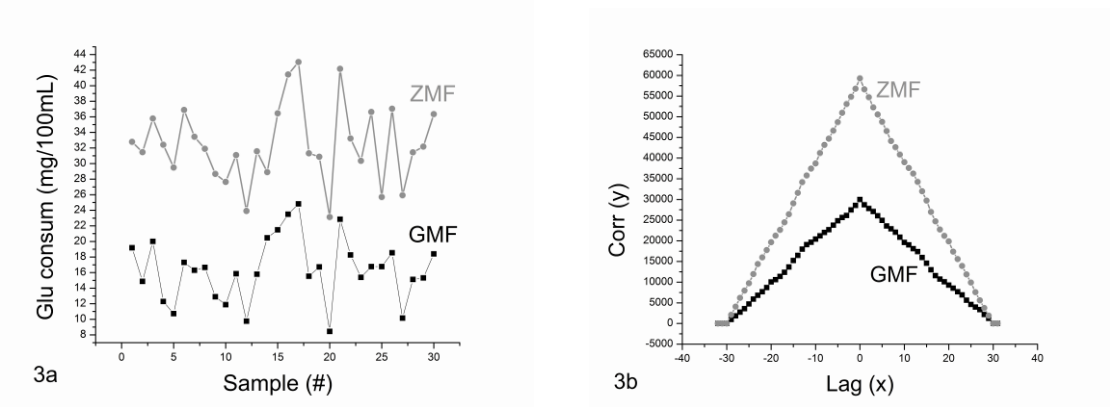

Fig.3 Glu consumption after 72h exposure in ZMF (gray), and GMF (black) correlation, for all semen samples (3a). Correlation result for ZMF (gray) and GMF (black) exposed samples (3b).

There is a good correlation between initial RP cells proportion and Glu consumption in ZMF (Corr (y) = 59377.04), and GMF (Corr (y) = 29975.4) (Fig.3).

Since during our experiments Ap index is less than 15 (minor magnetic storm indice level), our experiments were performed in quiet geomagnetic activity (Fig.4). This indicates that our experimental conditions were not influenced by magnetic storms. The interval of the experiment is delimited in the plot by arrows.

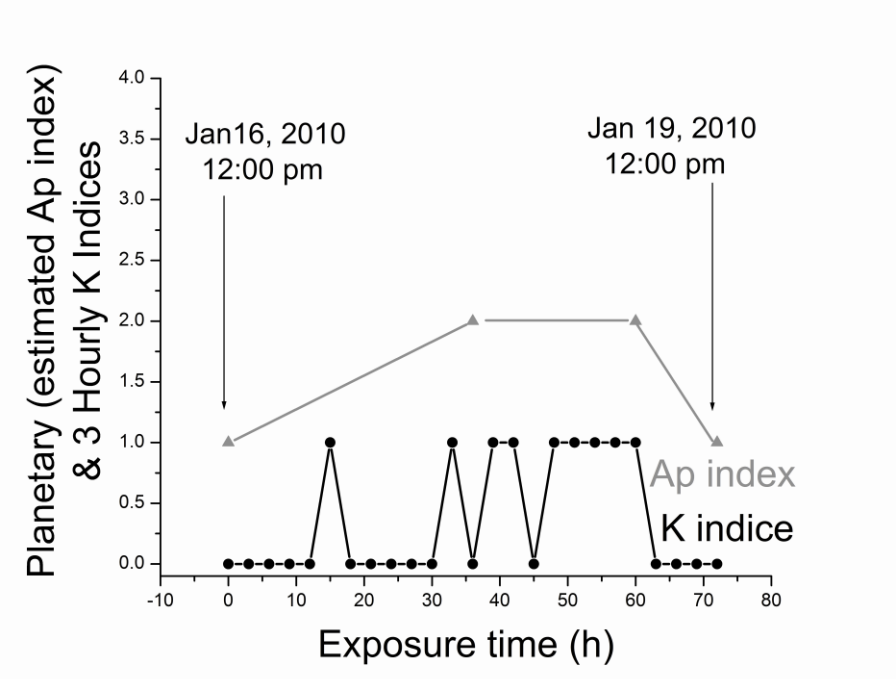

Fig.4 Geomagnetic activity in the period of spermatozoa cells exposed in ZMF and GMF.

We concluded that the effect recorded in ZMF conditions is due to the absence of static geomagnetic field.

The initial FFT correlation amplitude, between RP cells proportion and Glu concentration is 79958.8, for a corresponding power of 9.98E7, at zero Hz (Fig. 2b).

On the other hand, the good correlation between Glu consumption in ZMF, respective GMF, with RP cells proportion (Fig. 3), suggests that human spermatozoa Glu demand is mainly finalized to their motility. Similar results are reported, in GMF, for other mammalian species (MUKAI & al. [4]).

However, the correlation amplitude between RP cells proportion and Glu consumption is almost double in ZMF, comparing to GMF (1.98).

Results show that after 72 hours exposure in ZMF, average semen sample Glu consumption is 32.4±4.9 mg/100mL, while after 72 hours exposure in GMF average semen sample Glu consumption is 16.4±4.1 mg/100mL (Tab.4). After 5h ZMF exposure human semen exhibited an average 10.23% increase in the percentage of RP sperm (66.43% from 60.26%, $P < 0.05$; Tab.1), relative to a 3.31% decline for matched GMF controls (58.26% from 60.26%, $P < 0.05$; Tab.1). The results in these studies [Tab.1, Tab.3] were compared to those previously published (BARAK & al. [1], MAHAVEN & al. [2], WILLIAMS & al. [5],

MIKI & al. [7], TRUTA & al. [8]) to confirm the importance of glucose, as major bioenergetical substrate, for human sperm motility.

Conclussions

At ZMF exposure for 5 h, human sperm motility was significantly increased. This suggests that a single 5h ZMF in vitro exposure, can result in a measurable change in sperm motility in humans, and this implies direct affects on sperm quality and fertility estimates.

Further, we demonstrated that human healthy semen Glu metabolic consumption increases significantly in ZMF conditions. Coupling, motility increases in ZMF. The good

correlation between motility increase, and human spermatozoa Glu consumption increase, suggest that the energy resulted from breaking up the Glu is used mostly for spermatozoa movement.

References

1. Y. BARAK, S. GOLDMAN, Y. GONEN, Z. NEVO, B. BARTOOV, A. KOGOWSKI, Does glucose affect fertilization, development and pregnancy rates of human in-vitro fertilized oocytes?, *Hum. Reprod.*, 13, 4203-4211, (1998)
2. M.M. MAHAVEN, M.M. MILLER, D.M. MOUTOS, Absence of glucose decreases human fertilization and sperm movement characteristics in vitro, *Hum. Reprod.*, 12, 119-123, (1997)
3. K.A. KNUTH, E. NIESCHLANG, Comparison of computerized semen analysis with the conventional procedure in 322 patients, *Fertility and Sterility*, 49, 881–885, (1988)
4. C. MUKAI AND M. OKUNO, Glycolysis Plays a Major Role for Adenosine Triphosphate Supplementation in Mouse Sperm Flagellar Movement, *Biol. Reproduction*, 71 (2), 540-547 (2004)
5. A. C. WILLIAMS, W.C. FORD, The role of glucose in supporting motility and capacitation in human spermatozoa, *J. Androl*., 22, 680-695, (2001)
6. D. H. OWEN, D. F. KATZ, A Review of the Physical and Chemical Properties of Human Semen and the Formulation of a Semen Simulant, *Journal of Andrology*, 26 (4), 459-471, (2005)
7. K. MIKI, W. QU, E. H. GOULDING, W. D. WILLIS, D.O. BUNCH, L.F. STRADER, S. D. PERREAULT, E. M. EDDY, D. A. O'BRIEN, Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility, *PNAS*, 101 (47), 16501-16506 (2004)
8. Z. TRUTA, S. NEAMTU, V. V. MORARIU, Zero magnetic field influence on in vitro human spermatozoa, *R. J. Biophysics*, 15 (1-4), 73-77, (2005)
9. Z.TRUTA, S. LERINTIU, M. GARLOVANU, V.V. MORARIU, Zero magnetic field influence on male reproductive cells progressive motility distribution, *Sudia Universitatis Babes-Bolyai, Physyca, LI*, (1), 39-43, (2006)
10. L.I. DE-KUN, B. YAN, Z. LI, E. GAO, M. MIAO, D. GONG, X. WENG, J.R. FERBER, W. YUAN, Exposure to magnetic fields and the risk of poor sperm quality, *Reproductive toxicology* , 29, 1, 86-92, (2010)
11. T.H. LEE, C.H. LIU, Y. T. SHIH, H.M. TSAO, C.C. HUANG, H.H. CHEN, M.S. LEE, Magnetic-activated cell sorting for sperm preparation reduces spermatozoa with apoptotic markers and improves the acrosome reaction in couples with unexplained infertility, *Human Reproduction*, 25(4), 839-846, (2010)
12. V.N. BINHI, Stochastic dynamics of magnetosomes and a mechanism of biological orientation in the geomagnetic field., *Bioelectromagnetics*, 27, (1), 58-63, (2006)
13. MAKLER, A new multiple exposure photography method for objective spermatozoal motility determination, *Fertil. Steril*., 30, 192-199, (1978)
14. WORLD HEALTH ORGANIZATION, *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction*, Cambridge University Press, Cambridge, 1224-1241, (2001)
15. Z. TRUTA, M. GARLOVANU, S. LERINTIU, R. MICU, A new method for human semen glucose concentration evaluation, *R. Biotechnological Letters*, 6, 5764-5772, (2010)
16. http://sec.noaa.gov/Data/index.html#indices.
17. P. IGHINADUWA AND B. IGHINADUWA, The Predictive Value of Glucose-fructose Ratio in Seminal Plasma, *World Applied Sciences Journal*, 2, 602- 605, (2007)