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BIOPHYSIC EFFECTS OF ELECTROMAGNETIC FIELDS ON HAPLOID CELLS

DOCTORAL THESIS SUMMARY

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BRIEF SUMMARY OF WORK

Presented study aims to investigate the effects of changing the Earth geomagnetic field (GMF) on motility parameters of human male fertility. Human male fertility has decreased considerably in the last century, and there are few studies to investigate the reasons why estimates of fertility decline in men. On the other hand GMF decreases and we are moving towards a period of change of the magnetic poles.

The transition will be a period of time when Earth will inevitably have zero magnetic field (ZMF). This doctoral thesis aims to study changes that occur in microscopic fertility parameters of male reproductive cells, when GMF is compensated and we gain ZMF conditions.

As these factors are vital to the natural process of reproduction, we decided to study them in terms of bioenergetics. For this we studied the relationship between the primary bioenergetics substrate (glucose) used by human haploid cells, and the resulting energy is used for mechanical movement of spermatozoa and spermatozoa methabolism. We considered the glucose consumption as the starting parameter, and as final parameters, the motility parameters of human male fertility. Mention that the results are illustrative, presenting in detail only few samples for each parameter studied.

The paper is structured in five chapters. In the first part we present a brief overview of the current research in this field. In the second part we present in detail the methods used in dealing with this topic, focusing on our new developed method. In the third part we present the influences of magnetic fields on human cells, with emphasis on their results and possible explanations of the phenomena investigated. In fourth part we present in detail ZMF influences on motility parameters of human male fertility. We discussed in detail the possible justifications of the results, we correlated the results and we have presented a synthetic view. We also presented in detail rules and methodology to be followed to return and reproduce the experiments presented, avoiding unwanted errors, by anyone interested in this topic. In the fifth part we present the clear conclusions from our experiments.

In order not to destroy chemical concentration samples during glucose determinations, we planed to develop a noninvasive and nondestructive method. To this end, we performed preliminary measurements to determine a method to give results with same errors as the latest chemical methods world wide currently accepted. Preliminary measurements have shown that Raman spectroscopy gives a coefficient of determination wich is not acceptable to measure glucose concentrations in human seminal fluid.

We chose UV-VIS spectroscopy as best, with a coefficient of determination acceptable for our purpose. To the best of our knowledge at this time, our study is performed in world premiere. Our own method of study was developed and validated as required in this field, and gives more flexibility in specific areas of research.

Measurements have shown that human sperm uses more glucose in ZMF compared to GMF. Also our results show that human sperm motility parameters, currently used to characterize human male fertility, changes in ZMF exposure values compared with GMF as control exposure.

Statistical correlations between study parameters were made and the results considered in detail. From the synthetic study of these correlations, it is clear that ZMF is stimulating human sperm motility compared to GMF, and significantly increases the estimates of male fertility.

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ORIGINAL CONTRIBUTION

The data presented in this paper are original and were published as follows:

Articles published in ISI journals:

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Truța Z., Truța M., Micu R., Zero magnetic field influence on human spermatozoa glucose consumption, accepted for publication by *Romanian Biotechnological Letters*, articol nr.313, in vol 16 (5) (2011).

Articles published in journals indexed BDI:

Truța Z., Neamțu S., Morariu V. V., Zero magnetic field influence on in vitro human spermatozoa cells behavior. *Rom.J.Biophysics* 15:73-77 (2005)

Truța Z., Lerințiu S., Gârlovanu M., Morariu V. V., Zero magnetic field influence on male reproductive cells progressive motility distribution, *Sudia Universitatis Babeş-Bolyai., Physica* LI 1:39-43 (2006)

Neamțu S., Truța Z., Boldizsar E., Pop C.V.L., V.V. Morariu, Cell motility in zero magnetic field, *Studia Universitatis Babeş-Bolyai, Physica*, (2005) L(4b): 697-700

Conferences:

Truța Z., Neamțu S., Morariu V. V., The influence of zero magnetic field on in vitro human spermatozoa cells behavior, Advanced biomaterials and biophysical techniques, may 26-28,2005, 8thNational Biophysics Conference (2005)

Truța Z., Neamțu S., Morariu V. V., Spermatozoa aging is slowed down in ZMF, Isotopic and molecular processes, Fourth PIM Conference, Cluj-Napoca, sept.22-24, (2005)

Truța Z., Lerințiu S., Gârlovanu M., Morariu V. V., Human haploid male germ cells trajectory in zero magnetic fields. Fifth PIM Conference, Cluj-Napoca, sept.20-22, p. 250 (2007)

Truța Z., Lerințiu S., Gârlovanu M., Micu R., Morariu V. V., Is zero magnetic field influencing human spermatozoa dynein molecular motor? 6th EBSA Congress, Imperial College, London, UK, European Biophysics Journal 36 (1) S197 (2007)

THEORICAL NOTIONS AND INTRODUCTORY

HUMAN HAPLOID CELLS

Van Beneden discovered in 1883 that the gametes have a small number of chromosomes. Chromosomes are groups that are generated in the cell nucleus during division [WHO, 2001]. Only in 1956 it was determined that the final number of chromosomes in humans is 46, and the process by which the number of chromosomes is halved is called meiotic division.

Cells bearing only half the genetic information, as having only 23 chromosomes, are called haploid cells. Human reproductive cells, gametes are haploid cells (Fig.1). Human sperm cell is carrying half of the genetic information of human male body. It is generated by dividing in the seminiferous tubes of the testis.

Human sperm is about 50µm long and consists of two morphologically and functionally distinct regions, head and tail, which are closed in the same membrane (Fig. 1). The main sperm functions are to deliver the haploid set of chromosomes and fertilization of the egg, leading to activation of the zygote development process. This haploid genetic information is condensed in the nucleus located in the sperm head. Sperm tail is composed of microtubules, two singular inside, surrounded by nine double microtubules outside.

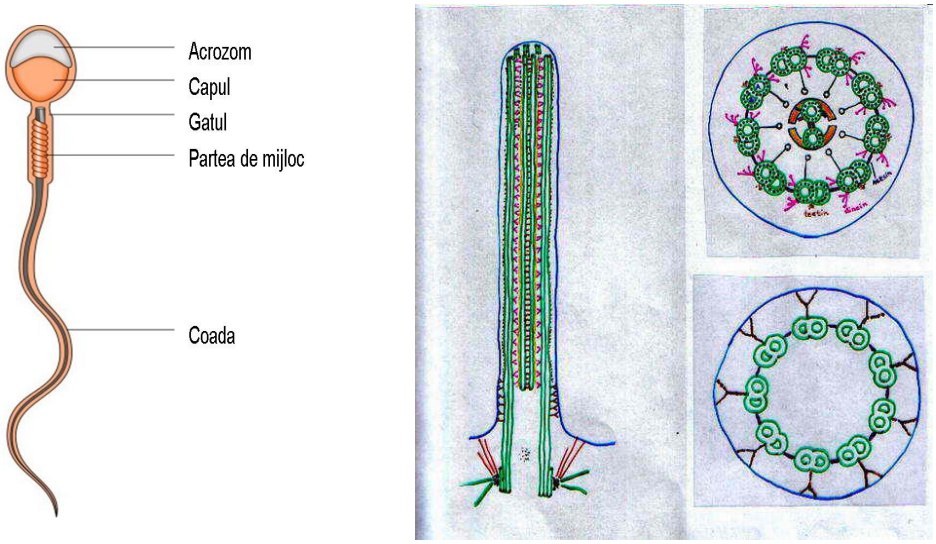

Fig. 1 The basic structure of a human spermatozoa and human sperm tail section view

In front microtubules are surrounded by mitochondria, which is collecting nutrients from the environment. Some of these nutrients from the seminal fluid, are converted into adenosine triphosphate (ATP), the bio-fuel used to move the tail.

HUMAN SPERMATOZOA ENERGETIC CONSIDERATIONS

The most important extracellular nutrients that human sperm uses are glucose and fructose. Glucose is the energy substrate used for biochemical chain reactions that occur in glycolysis, and fructose for fructolysis. The final product of fructolysis in human sperm, is lactic acid. This structure allows the interconversion of ATP and ADP (adenosine diphosphate) between fibrous layers, where ATP is produced by glycolysis, and axonemal dynein ATPase, where ATP is consumed [Wenli C. et.all, 2006]. Only D-glucose is biologically active, while its mirror image L-glucose can not be metabolized in the biochemical process known as glycolysis.

Glucose is a whitish substance, or as solid crystals or in aqueous solution [Kirschner and Woods, 2001]. Molecular formula is presented below as a cyclic form (Fig. 2). Glucose has the chemical formula ($C_6H_{12}O6$) and is found predominantly as cyclic at pH 7, and in aqueous solution in the form of light chain (acyl) and ring (cyclic) balanced. It is noted that although the chemical is a hexose ring, it contains only 5 carbon atoms, hence the name piranose.

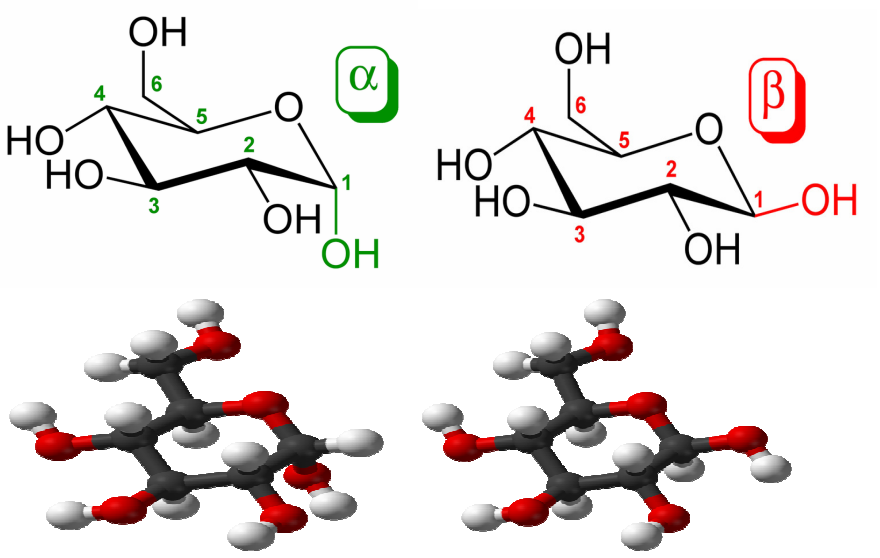

Fig. 2 Form and structure of cyclic α-D 3 glucose (left) and β-D glucose (right)

Molar mass of glucose is 180.16 g mol-1, density 1.54 g cm-3 and the melting point for α-D-glucose: 146 ° C and β-D-glucose: 150 ° C [Gailliot et al., 2007]. The data correspond to standard status (at 25 ° C, 100 kPa). Glycolysis can produce anaerobic, two molecules of ATP from each glucose molecule. With the oxidation of glucose to carbon dioxide, a process known as cellular respiration, one molecule of glucose releases energy by splitting full to produce 36 molecules of ATP [Lodish et al., 2004].

CONSUMPTION OF ADENOSINE TRIPHOSPHATE (ATP). MECHANICAL ENERGY PRODUCTION

According to standard status (at 25 ° C, 100 kPa) ATP has the chemical molecular formula $C_{10}H_{16}N_5O_{13}P_3$ and molar mass 507.181 g / mol. ATP produced is used for human sperm to produce mechanical motion. Gajewsky and his collaborators have shown that this hydrolysis is released amounts of energy depending on the type of chemical bond that cleaves. If G˚ is Gibbs free energy, we have [Gajewski et al., 1986] :

$ATP + H_2O \rightarrow ADP + Pi + H^+$ **ΔG° = - 30.54 kJ/mol (–7.3 kcal/mol)** **[1]**

$ATP + H_2O \rightarrow AMP + PPi + H^+$ **ΔG° = - 45.6 kJ/mol (–10.9 kcal/mol)** **[2]**

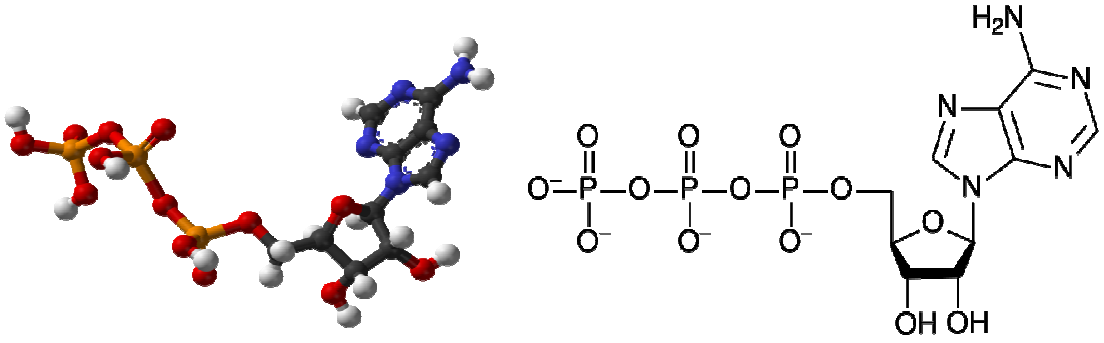

Fig. 3 Chemical molecular 3D structure of ATP

Glucose consumption in the main part of the tail is correlated with biochemical and quantitative production of biological fuel ATP. 200 pmol ATP are produced per 10^6 sperm in the absence of glucose, and about 1000 pmol ATP per 10^6 sperm in the presence of glucose [Mukai C et al., 2004]. Note that ATP production is 5 times higher in the presence of glucose. Therefore sperm using glucose as a primary source for producing the bioenergetical fuel ATP.

Once produced, ATP is consumed by axonemal dynein catalyst ATPase [Cao W et al., 2006]. ATPase are a class of enzymes that catalyze the decomposition of ATP into adenosine

diphosphate (ADP) and a free phosphate ion. This dephosphorylation produce energy, which in our case is mainly used for sliding of microtubules (MT) of haploid cell tail. Relative microtubule sliding, exterior form interior, generates a bending movement which helps produce a typical swimming movement (Fig.1). ATP and its analogs stimulate sperm motility [Romac P et al., 2004], and extracellular ATP increases fertilization potential and motility [Rossato M et al., 1999]. The sliding microtubule are stimulated from dynein ATPase, and the dynein arms are interconnected to obtain a movement of the tail. This sliding of microtubules in sperm are typical for both mammals and those of echinoderms [Ishijima did et.all S., 2006]. Dynein ATPase, are the basic component of molecular motors in cells of germination, and they require ATP as energy source [J. Du et al., 1994].

Central pair of MT is coupled mechanically with the 9 MT from exterior, with proteins thet become stable conformation through evolution. This coupling helps human sperm flagellate type 9 + 2 to bend and disengage power to generate beats, alternating with recovery movements [Yang C et al., 2008].

STANDARD PARAMETERS FOR STUDY OF HAPLOID CELLS MOVEMENT

Once released sperm behave as a complex eukaryotic cell, capable of producing and consuming energy, thus ensuring a steady equilibrium with the environment. To characterize the motility, viability and sperm characteristics a group of parameters were defined.

Liquefaction time is a parameter closely related to the viscosity of semen, and liquefaction is caused at 10-30 minutes after ejaculation by proteolytic enzymes from the prostate (protelase and amylase).

Volume and sperm concentration, ***glucose, fructose concentration and acidity*** are macroscopic parameters that give information about the quality of the sample and alkalinity. A normal sample has a pH between 7.2 - 8.0. Microscopic parameters are usually closely related to the number and shape of sperm. A normal sample has a concentration of at least 20 million sperm / ml, with a total of at least 40 million cells per sample ejaculate, of which at least 50% are motile (moving). The total number of leukocytes should be more than one million [Jequier AM Crish & J.P., 1986]. For a normal sample with normal morphology, the proportion of cells rapidly progressive (the average speed over 25 μm/s) determines the quality and fertility estimates [Derek H. Owen & David F. Katz †, 2005].

Sperm motility can be characterized by a specific group of parameters that can be determined from recording and sequential photographing cell trajectories (Fig.4).

VCL, ***curvilinear velocity*** is defined as the numerical equal to the distance traversed between two points, following the curvilinear path, per unit of time (Fig.4).

VAP, ***straight line velocity*** is defined as numerically equal to the distance traversed between two points in a straight line, per unit of time. Both speeds are measured in μm/s to be more convenient, as the real scale corresponding for human sperm specific movement.

LIN, ***linearity*** of the trajectory is defined as the ratio between the curvilinear distance and the distance traversed in a straight line following the current trajectory of movement for the cell to travel between two points considered (Figure 4). Therefore we see that LIN can be expressed in percentages, or often by a number in the range [0,1].

ALH, ***amplitude of lateral head displacement*** is defined as the average maximum travel distance of the sperm head, measured from the curvilinear path that runs through the center of gravity of the cell in its movement between two points. It is convenient to express this distance in μm, according to real scale specific for human sperm movement [Ginsburg KA et al, 1988].

BCF, is the ***beat cross frequency***.

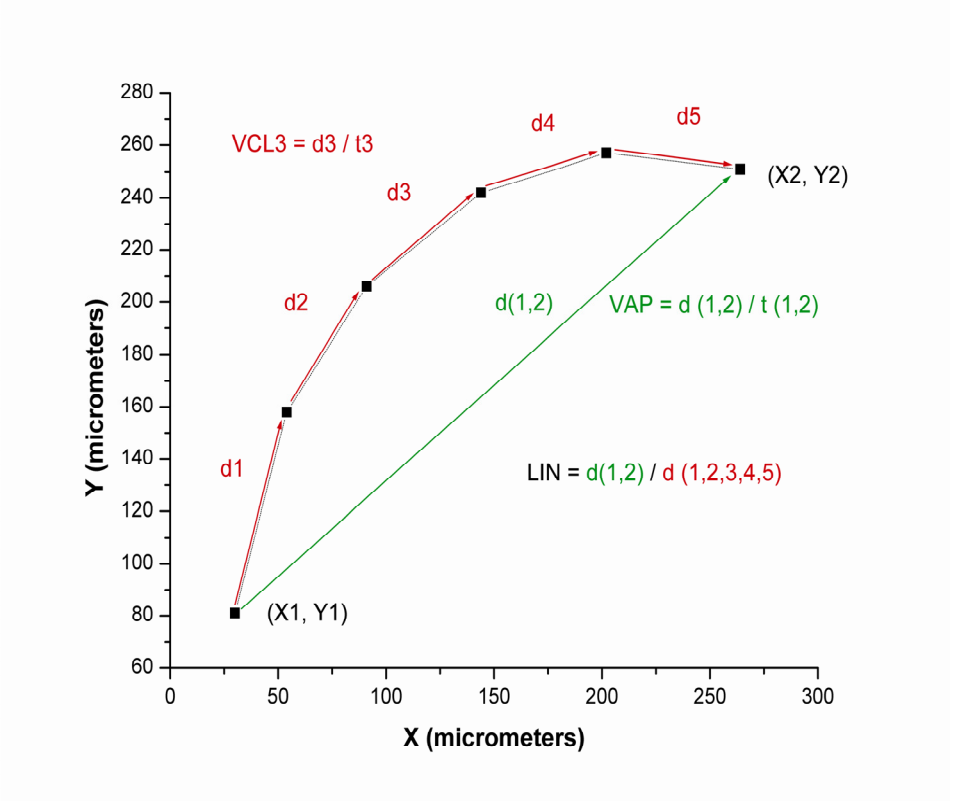

Fig. 4 Dividing the trajectory in portions approximately straight, and defining curvilineare velocity (VCL), straight line velocity (VAP) and linearity of trajectory (LIN)

EARTH GEOMAGNETIC FIELD (GMF)

A particular case of stationary field of interest in this case is the Earth's geomagnetic field. We often refer to magnetic flux density as the vector module B (magnetic induction) to characterize the magnetic field. Earth's magnetic field (and magnetic field surface) is in good approximation a dipole magnetic with a pole near the north pole, and a pole near the south pole. Earth's core temperature is above 1,043K, the Curie temperature for which the magnetic moments are disordered in iron, and therefore the local contribution to the Earth GMF can not be explained by the orientation of magnetic moments.

GMF is caused by currents in the outer shell of the Earth's liquid rather than magnetic dipole arrangement. Induced electric currents generate magnetic fields in the ionosphere when the atmosphere is closer to the Sun, causing daily alterations which can be deflecting up to a degree GMF [Herndon JM, 2003].

Earth's magnetic field changes direction on average every 250,000 years. The last such event has not complied with this periodicity. It was determined on the basis of basalt lava, in most parts of the Earth, and held 780,000 years ago. From the first GMF flux density measurement, made by Carl Friedrich Gauss GMF in 1835, to the present GMF decreased by about 5% [V. Courtillot and JL Le Mouel, 1988]. These statistics show that we are moving towards a time when Earth's magnetic field polarity will change. In the future, for a period of time there will inevitably be zero magnetic field on Earth (ZMF).

THEORETICAL BASIS FOR HUMAN MALE HAPLOID CELLS STUDY IN ZERO MAGNETIC FIELDS (ZMF)

Explanation of ZMF influence on human sperm motility was suggested in reports [Truța Z et al. 2005, Truța Z et al. 2006, Truța Z et al., 2011]. To understand this influence, we need to know that static magnetic field is inducing residual dipole coupling of amino groups that are in the structure of nucleic acids [Ying J et al., 2007]. On the other hand, human sperm contain nucleic acids in DNA, condensed in the head.

Sperm proteins contained in human dZnein are diamagnetic elements (static magnetic field easily rejected) [Alberts B et al., 2002]. As a result of diamagnetic anisotropy of proteins and nucleic acids in human sperm, these macromolecular structures will tend to orient in a magnetic field. There are studies that show that sperm of other mammals are moving in static magnetic field [T Takeuchi et al., 2002]. Therefore, compensating GMF we are canceling several potential disturbing factors of free movement and orientation of sperm,

and, theoretically, they will move better. This assumption was verified experimentally for human sperm and first reported in 2005 [Truța Z et al., 2005].

Biological fuel ATP is consumed by both haploid cells to produce mechanical energy and the energy needs of metabolic reactions. Extracellular ATP increases fertilization potential and sperm motility [Rossato M et al., 1999]. ATP to generate movement is consumed with axonemal dynein catalyst ATPase [Cao W et al., 2006]. When biological fuel ATP concentration is higher in seminal fluid, and cell motilitii increases. Bioenergetic behavior of human male haploid cells can be understood if the primary bioenergetic substrate glucose correlates with the production of biological fuel ATP and mechanical motion through the consumption of ATP.

A special case is ZMF conditions, which is why we designed this study, choosing haploid cells, as one of the most complex human generated cells. We suggested the explaination of the primary influence of ZMF on human sperm [Truta Z et al. 2005; Truța Z et al., 2006] having regard to complete a study that took into account glucose as starting parameter bioenergetics and the motility parameters as the final parameters of the study . Our study was designed to determine the effects of glucose ZMF consumption of human spermatozoa and the motility parameters, and viability of these cells, the main parameters in predicting male fertility.

EXPERIMENTAL METHODS

SAMPLES ACQUISITION AND HANDLING PROTOCOL

Biological samples used were donated to the IVF laboratory at the Gynecology Clinic of Cluj. Samples were obtained by ejaculation following the procedure recommended by the World Health Organization [WHO, 2001]. Each sample was observed microscopically, counted, marked and divided into two samples. A sample was exposed to ZMF and the other in GMF as control sample.

For each sample 10 measurements were made by of quantities of 5µl. The process of categorization was done by counting the Makler chamber. To have the same temperature conditions have kept the sample and control sample in the same room (there were small variations in temperature, 22 ± 0.3°C). The maximum exposure was considered 72 hours. After counting measurements, motility, and record PC spectrophotometry, all samples were destroyed following the protocol of the destruction of biological samples.

No evidence has been used for magnetic field exposure in other studies, IVF or insemination. Makler counting method was used for the sperm counting [Makler A et al., 1978]. We used specific notation known for cell motility (rapid progressive, slow progressive cells, and immobile cells %) for characterization of all samples used in the study. The percentage of cells rapidly progressive (RP) is determined by dividing the number of cells that move at a speed higher VAP 25μm/s, to the total number of cells. Similar concentration of cells slow progressive (SP) is determined by dividing the number of cells moving at a speed of less than 25μm / s, the total number of cells [WHO, 2001].

GLUCOSE OXIDASE METHOD (GO)

GO method is based on reactions:

$$\text{Glu} + O_2 \xrightarrow{\text{Glu oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + O_2$$

$$O_2 + \text{Chromogenic } O_2 \text{ acceptor} \longrightarrow \text{Colored complex}$$

Chromogenic O_2 acceptor reacts with peroxide chromogenic product, and forming a color proportional to the concentration of glucose in the sample of semen. Note that to verify the chromogenic oxygen acceptor we originally used pure glucose samples diluted to known concentrations. For sample preparation was used Kern balance with 1mg error measure. A laborant and a biologist performed the same tests separately, and the results were compared, and shown to be the same.

ULTRAVIOLET-VISIBLE SPECTROSCOPIC METHODS (UV-VIS)

UV-VIS spectrophotometry is a fundamental analytical techniques, together with the specific accessories, is used in laboratories for measurements of absorption and transmission in all areas of application. Spectrophotometric method measures the relative components, compared to absolute components that are difficult to measure, and depend upon the measurement technique used. We used a UV-VIS spectrophotometer JASCO 530 with two identical cuves, one for the sample and one for reference. To avoid errors caused by distilled water in the reference cuves, we obtained UV-VIS spectrum of distilled water and we allways extracted the differentiation of spectra of interest.

Spectrofotometria UV-VIS este o tehnică analitică fundamentală, care împreună cu accesoriile specifice, este folosită în laboratoare pentru măsurători de absorbție şi transmisie în toate domeniile de aplicație. Metodele spectrofotometrice măsoară componentele relative, în comparație cu componentele absolute, care sunt dificil de măsurat, şi depind de tehnica de măsurare folosită. Am folosit un spectrofotometru UV-VIS JASCO 530 cu două cuve identice, una pentru probă şi una pentru referință, la care am raportat. Pentru a evita erorile cauzate de apa distilată din cuva de referință, am obținut spectrul UV-VIS al apei distilate şi l-am extras prin diferențiere din spectrele de interes.

COMPUTER ASSISTED SPERM ANALYSIS METHOD (CASA), MOTILITY AND VELOCITY PARAMETERS CALCULATION

To determine the straight line velocity (VAP) and curvilinear velocity (VCL), we used the CASA as a new efficient method. Usual parameters were determined mediators of motility and velocity standard [Fletcher DA and Theriot JA, 2004]. For verification we determined the scale of measurement in pixels for the display monitor used, with Makler grid markings (620:1 on the Toshiba and zoom 2480:1, 1358:1 on the Sony).

VAP was determined for all sperm samples for at least 10 consecutive photo lifting (20-30 cells) from at least five different locations of Makler counting chamber, for one and each sample at a time of measurement. This method gives a total of 100-150 cells used for a measuring set.

View was made simultaneously on a Emerson TV, and with an analog-digital tuner PCTV from the same CCD camera, on the Toshiba computer. Each set of measurements were mediated and statistical errors calculated. Standard errors of deviation were calculated and the results presented are significant at $p < 0.05$ level.

GMF COMPENSATION AND SAMPLES EXPOSURE

We built a device to create continuous magnetic field, consists of a set of coils Helmoltz (Fig.5), and two rectangular bases from extruded polystyrene as support.

The base outside of the coils (base support) was built to be kept horizontal and within certain limits to be placed conveniently, marked in relation to space exposure of samples chosen.

Inner coils base (the exposure base) was elevated by a set of polystyrene estrudat supports to ensure stability, amid evidence about the geometric parallelism between the support and exposure. Interior base elevation was made by superimposing several rectangular non-magnetic bases, of the same material.

Coil planes can be inclined at an angle greater than 45 hexagesimale degrees. In combination with the rotating support base and reversing the current in the coils, we can create a continuously magnetic field to provide any desired spatial orientation. Once we get the desired orientation, the support base is marked over the place chosen for exposure.

We used a voltage source with Nokia rectifier AC 220V and a filter power network for Europe type, with an output in 12V DC and 0.5A. We introduced in the output circuit a rheostat to adjust the current passing through the coils. DC voltage terminals can be swapped, thus meaning changing the coil sense of current, meaning changing the direction of the magnetic field created by coils.

Along the marks of the exposure surface, we successive measured over the area of interest. A magnetic map of the space was determined over the exposure surface, in coils, with a Fluxmaster device, measuring the vertical and horizontal component of magnetic induction. The magnetic flux density obtained with the exposure apparatus built by us, gives a maximum horizontal or vertical variation ranging 300nT/25mm.

This variation in surface of magnetic flux density of 60nT/5mm is acceptable because the eprubetes used for sample exposure had a diameter of 5mm. Each sample was exposed to the minimum volume of 10μL.

Therefore the magnetic field flux density variation between samples exposed compact is significantly lower than the proposed error for compensation magnetic field (150nT). Field homogeneity achieved gives errors less than 0.3% of the value of magnetic flux density value at the exposure location (50μT), errors that we have not exceeded for the exposure of the samples.

We wanted that the cotidiene random influences to be identical for samples exposed in ZMF and exposed in GMF, so the relative differences we record between samples, are not due to electromagnetic radiation (EMF) or low frequency electromagnetic radiation (ELF). We've reassured so that differences arising from the evidence presented between GMF and ZMF exposed samples, are due to GMF field cancellation effect, rather than deterring common everyday influences variable fields. Were also eliminated all possible sources of EMF radiation, ELF, radio and TV radiation near the exposure site.

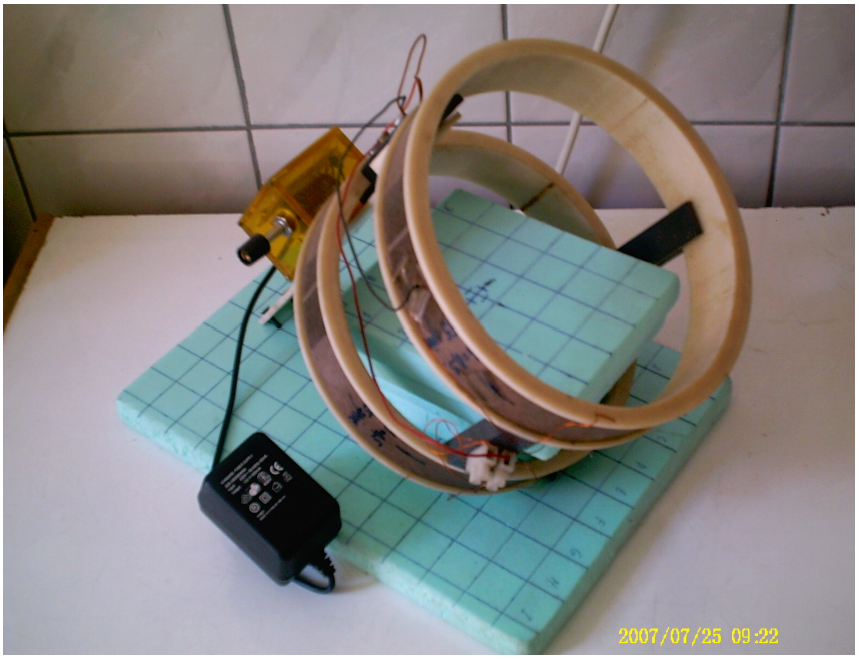

Fig.5 Device for compensating Earth's geomagnetic field GMF

STATISTICAL METHODS AND STATISTICAL RESULTS ANALYSIS

Repeated measurements were analyzed using statistical software SPSS 13.0 for Windows (SPSS Inc., Chicago, Ill.) and One-way ANOVA for Origin 6.1. Results from counts Makler and Makler undiluted at 1:1 were tested systematically using Intraclass Correlation Coefficients absolute correlation (ICC), and the double-effect model. Repeated for each sample should give the same values. The ICC value is close to 1, the method is more reliable. Coefficients of variation (CV) were also calculated to determine the initial results from the average spread for each method.

To identify glucose UV-VIS peak (GUVP) from spectrum of human seminal fluid samples, we chose three statistical tests: Independent t-test, One-way ANOVA, and Multiple Regression. Tests can be applied to two independent data sets with a normal distribution. Independent t-test test was used as test two population t-test. All three tests can be used to determine whether or not data sets have the same kind of variation (or distribution from case to case), and if or not they have the same mean value.

The column "Prob> F" methods use gives the possibility to obtain statistical F value greater than that listed for regression by pure chance only. In normal applications this number is 0.05 or less. It is widely accepted standard in scientific papers. R^2statistic measures the variability in a linear model data to which applies (and can be explain with). R^2 takes values

from 0 to 1. In all tests and statistical analysis performed we imposed the minimum level of confidence of 95%. For specific Raman peaks control we used to identify substrates, we employed two tests listed at a time. For motility measurements of fertility parameters (VAP, VCL, LIN, ALH, BCF) results are statistically significant at $p < 0.05$.

MAGNETIC FIELDS EFFECTS ON CELLS

THE MECHANISMS OF INTERACTION

Mechanisms of interaction of static magnetic field with living cells can be partially explained by this date in several ways [WHO, 2006]:

- Electrodynamic interaction with ionic conduction currents. Ionic conduction currents interact with magnetic field through the Lorentz forces, exerted on moving charge carriers. These effects lead to the induction of electrical currents and potential changes. Electric potential changes are generally associated with ventricular inhibitor contractions.

- Magnetomechanic effects, including the orientation of anisotropic magnetic structures in static fields and ferromagnetic and paramagnetic materials, in translation field gradients.

- Magnetic field effects on electronic spin states of the chemical reactions biocomponents. Many classes of organic reactions can be significantly influenced by magnetic fields from 10 to 100mT as a result of the effects of electronic spin states of reaction intermediates. A pair of spins correlated radical can recombine and prevent a reaction product in two situations that can be influenced by magnetic field:

- a transition from a triplet state to singlet state.

- physical radicals must meet to react. This can inhibit enzymatic reactions, and therefore metabolic chain reactions.

Mechanisms of interaction of variable magnetic field (electromagnetic field) with living cells is explained on the basis of the erergy the wave carries. Emission frequency of a source is one that dictates the type of interaction with organic matter.

Electromagnetic radiation → Ionizing → γ, X, UV-high (Ionization compounds)

→ Non-Ionizing → low UV, VIS, IR (electronic excitation)

→ RF, MF, ELF, SF (induce currents)

At frequencies below RF frequencies amplitude modulated (AM) of about 10^6 Hz electromagnetic radiation is weak coupled with organic matter, so the effects due to extreme low frequency wave frequency (ELF) are lower. At this level we must take into account the static component of the field, and as a result and the mechanisms explained above [JE Moulder and KR Foster, 1995]. Magnetic fields are difficult to screen, they easily penetrate even through buildings, especially organic matter (in part, human body). In contrast, electric fields have little ability to penetrate buildings and organic matter.

Since the static electric fields do not penetrate deep in the human body is natural to believe that any biological effect related static fields must be due to static magnetostatic fields [Kowalczuk CI, 1991, Miller G, 1987]. The question is how much we are able to support from these radiation without irreversible health problems. American National Standards Institute (ANSI) has standardized the maximum dose of 0.4 W/kg specific absorption rate (SAR) [ANSI, 1982].

GENERAL ELECTROMAGNETIC RADIATION (EMF) EFFECTS ON HAPLOID CELLS

Mobile phones are indispensable today. They emit electromagnetic radiation during operation that may be harmful to cells, particularly for male haploid cells. Industrial speaker phones produce a magnetic flux density of 0.3-1.0mT at surface. There are reports that found significant increases in motility parameters of pulsating magnetic field exposure in the form of square pulses, at 50 Hz and intensity of 5mT.

At magnetic flux density of only 2.5mT, for the same type of influences, the effects were not significant [Iorio R et al., 2006]. ELF did not induce chromosomal aberrations or mutations in the nucleic acids [Hiroyuki T et al., 1998].

Studies have considered human sperm have determined that both ELF and RF radiation causes reduced motility and fertilizing capacity of the haploid cells [Makler A, 1980, De Vita R, 1995]. Hoever waves with a frequency of 50Hz does not affect structural morphology of human cells in vitro [Supino R et al., 2001].

STATIC MAGNETIC FIELDS (SF) EFFECTS ON HAPLOID CELLS

Static magnetic fields are characterized by the strong magnetic field H <A/m> and the magnetic flux density B, <T= Wb/m^2> approximately constant over time. The Earth produces

a magnetic field of 35μT at the equator and 70μT at the poles. Ground electric field intensity is about 130V/m at sea level, and 45V/m at 1,000 m altitude.

Biological effects are influenced by the magnetic field at the actual applicable exposure. Computational studies are linking external static magnetic field and electric field with induced currents due to internal movement of organic matter, in that magnetic field. In the case of human spermatogenesis and sperm in vivo, be cause thenapplying magnetic field in the testis has approximately the same order of magnitude as the external magnetic field, makes the biological effects to be more pronounced in these kind of cells.

The highest static magnetic fields, to whom the public is exposed, are generated by magnetic resonance image systems (MRI). A computational theory calculation shows that in this case the public is exposed to a static magnetic field flux density of 1.5 - 4T [Kowalczuk CI, 1991, Stuchly MA, 1986]. Aluminum production workers are exposed to fields of 5 - 15mT [NIOSH, 1994].

Another type of biological effect is sperm orientation in a static magnetic field. There are works where orientation of male haploid cells in mammals (bull) is reported 100%, to a static magnetic field slightly below 1T. Biological effect of static magnetic field orientation of male haploid cells is more pronounced compared with erythrocytes and most types of somatic cells [Emura R et al., 2001].

Another study shows that exposing sperm mammals (mice in this case), to 0.7 T between 1 and 24 hours over 35 days, are growing more sperm head abnormalities, but there are no significant changes at the tail structure [Tablado et al. 1998]. All mice in an exposure of only 30min/day had reduced testicular sperm [Narra et al., 1996]. Even exposure to sperm at 5nT increases abnormal sperm count [Asashima M et al., 1991].

For other types of animals there are studies reporting adverse effects in animals exposed to a field with a value of magnetic flux density of 20mT. At an exposure of only 30 min/day over two weeks, sperm count has changed drastically, up to 89.9%, and sperm motility decreased by 96.54% [Ramadan LA et al., 2002].

GMF EFFECTS ON HAPLOID CELLS

Terrestrial geomagnetic field (GMF) is an almost stationary magnetic field. Therefore all the effects described in the previous chapter are manifested in haploid cells, relative to the Earth's magnetic flux density.

GMF gives unceasing apply to people on Earth as opposed to artificial sources that affect only certain locations, and apply temporary. Artificial sources of strong magnetic field influences the mass public very rare.

GMF effect accumulates over time. Thus, although the body has learned to live in these environmental conditions, they can not be neglected, the more so as they change over time, and we must ask ourselves how the body responds to these changes.

Human sperm cell is anisotropic and asymmetric containing diamagnetic macromolecular structures (structural proteins and proteins forming the dynein molecular motor) and nucleic acids [Ying J et al. 2007; Alberts B et al., 2002]. These macromolecular structures tend to orient in a magnetic field, so they are influenced by the GMF field, which becomes a stress factor for these types of cell motility.

HUMAN SEMEN SPECTRAL BASE

Spectra were extracted from UV-VIS samples diluted 1:5, for all biochemical components with significant concentrations (> 5mg/100mL) from human seminal fluid [C. Mukai et al. 2004]. Serum samples prepared artificial components, were respected as found in the literature of the newest work of synthesis for field experiments we found at this time [C. Mukai et al. 2004]. It is noted that the limit of detection of biochemical components in human seminal fluid is 5mg/100mL. Relative absorption intensity (Abs) was measured with a spectrophotometer Jasco 530. We chose the optimal dilution of 1:5. Since some of the UV-VIS spectra are overlapping, we obtained spectra of all major components of seminal fluid [Owen DH, Katz DF, 2005]. We have eliminated the spectral base contributions by differentiation (Origin 6.1) from values, at the 267nm glucose UV-VIS peak (GUVP) wavelength.

Also some biochemical constituents of human seminal fluid have overlapping absorption bands with a well-defined UV-VIS spectrum, but at a wavelength of 267nm their spectral base does not affect the GUVP extraction.

Measurements aimed at GUVP at 267nm. Citrate present an example (Fig.6). The UV-VIS absorption band with the closest peak we found it was the experimental fructose. Fructose has a clear absorption band woth a peak at 286nm (Fig.6). Pure fructose is used to raise a spectrum chart clear, and is widely accepted in studies.

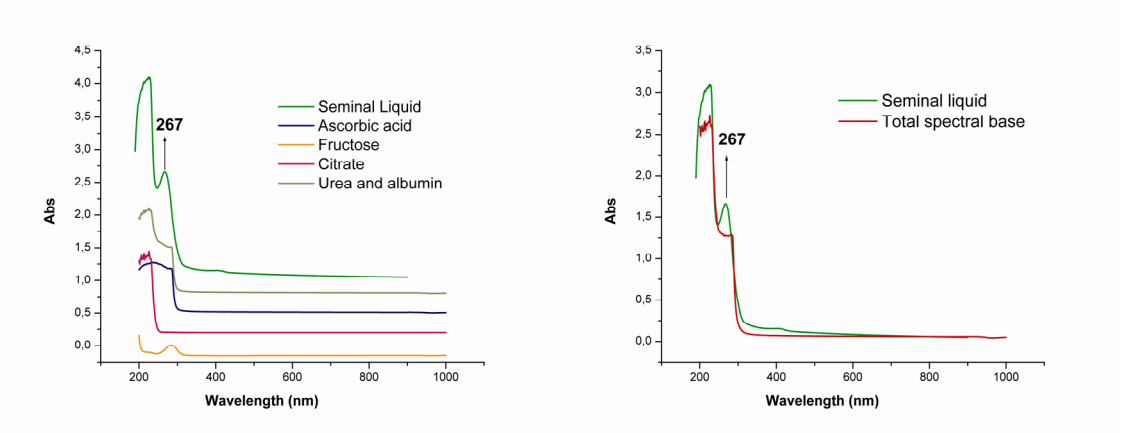

Fig.6 UV-VIS spectra of seminal fluid, and biochemical components of semen that have a significant contribution to the spectral base, diluted 1:5 (left). Total UV-VIS spectral base of human seminal fluid (72, 0, 28%) (red) determined graphically (Origin6.1), and UV-VIS spectrum of the same samples (green) at 1:5 dilution (right)

Simmilar as the mentioned peak results were obtained in advance not in semen, but in similar conditions of pH> 7 [Kubota H et al., 1976, Yordanov ND et al., 2004]. UV-VIS spectral base of human seminal fluid around 267nm, show only few significant spectral components: albumin, urea, ascorbic acid, citrate and fructose (Fig.6). Scale fitting of UV-VIS spectra of the biochemical components of human seminal fluid, which have a significant spectral contribution around 267nm, and the amount of their spectral (Origin 6.1), shows that the semen UV-VIS spectral base can be acceptable approximated (Fig.6).

We suggest that the difference between total UV-VIS spectrum of human seminal fluid (72, 0, 28%), and UV-VIS spectrum of the spectral base of the same samples at wavelengths below 250nm, is due to human seminal fluid content of other proteins. However they do not influence the graphical extraction of glucose peak at 267nm (Fig.6).

GLUCOSE INITIAL CONCENTRATION CALCULATION IN THE ANALYZED SAMPLES. A NEW OWN DEVELOPED METHOD BASED ON UV-VIS GLUCOSE PEAK (GUVP)

No other biochemical components of the seminal fluid, with the exception of glucose, has a UV-VIS absorption band around 267nm (see previous chapter). We present the UV-VIS absorption band with peak at 267nm clear glucose (Fig.7).

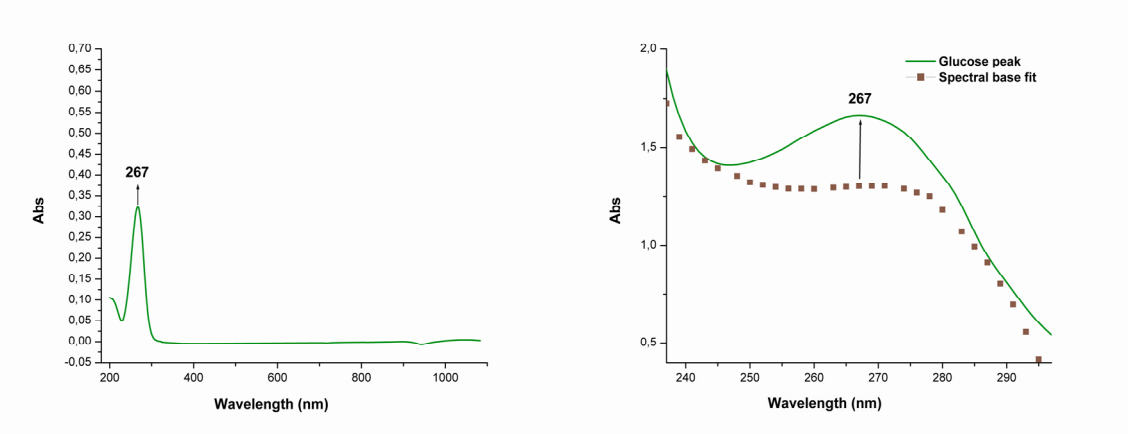

Fig.7 UV-VIS spectrum of a pure glucose solution concentration of 55 ± 1mg/100mL dilluted 1:5 in distilled water (left).Third grade polynomial regression (wine) corresponding to human seminal fluid spectral base for a healthy sample (72, 0, 28%) diluted 1: 5

Corresponding regression equation that approximates the best UV-VIS spectral base in the area of interest (230nm to 300nm) is:

$$\mathbf{Y = 452.71657 - 5.17716\ X + 0.01979\ X^2 - 2.51964E\text{-}5\ X^3} \qquad \mathbf{[3]}$$

Where **X** is the numerical value of the wavelength, and **Y** is the Abs value corresponding to the regression fit point by point.

From equation [3] and Fig.7 GUVP can be extracted simply using analysis of differentiation of Origin 6.1. To be sure the peak we measure is indeed GUVP peak, we compared and analyzed diluted 1:5 pure glucose peak (Fig.7), with the peak at 267nm extracted from the UV-VIS spectrum of the semen sample considered (Fig.7). The coefficient of determination resulted is 0963 (Tab.1).

Parameter/ Method	Independent t-Test	One-way ANOVA	Multiple Regression
p	0.27754	0.27754	0.027754
t	-1.10166	-	-
F	-	1.21364	1.21364
R^2 (COD)	-	-	0.96335
Prob>F	<0.0001	<0.0001	<0.0001
Final conclusion	Same mean	Same mean	Same mean

Tab.1 Comparison of the 267nm peak extracted from a sample of sperm (72, 0, 28%), and extracted GUVP from a UV-VIS spectrum of pure glucose concentration of 55 ± 1mg/100mL, diluted 1:5

:

From point by point comparison of the peak of pure glucose diluted 1:5 (Fig.7), with the peak at 267nm UV-VIS spectrum extracted from the semen sample considered (Fig.7), we clearly identified the GUVP in human semen (Tab.1).

To obtain a calibration graph of glucose concentration on the amount of relative absorption intensity of Abs, we measured the absorption at 267nm pure glucose, 5 different concentrations, following dilution in which I designed the study, 1:5 (Fig.8).

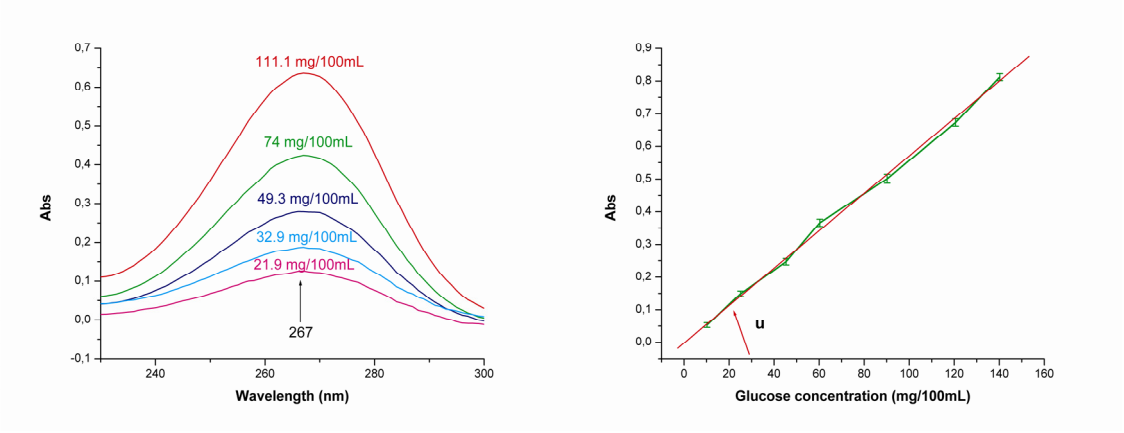

Fig.8 GUVP of pure glucose, diluted 1:5 at the concentrations shown (left). Fitting linear scale by plotting the resulting glucose Abs value as a function of glucose concentration at 267nm, diluted 1:5 (right)

We determined a calibration graph of relative absorbance of the sample depending on the concentration of glucose in the range of concentration from 10 ± to 140 ± 1mg/100mL 1mg/100mL. The calibration graph result linear fit equation is:

y = (5.7211 E-3) x + 3.6226E-5 **[4]**

Where **x** is the value of glucose concentration, and **y** is the value of the relative peak intensity of glucose absorption from 267nm for the same sample. Note that we used the concentration units specific widely accepted for these types of tests, at 1:5 dilution without changing any share or value Abs. If we neglect the second factor in the equation [4], the calibration graph (Fig.8) results:

x = y ctg u **[5]**

In equation [4] the first coefficient fitting error is ± 0.02413 E-3.

The coefficient of determination of the linear fit calibration graph is R^2 = 0.99864, and the calibration graph is significant at p <0.05 (Fig.8). In equation [5] **u** is defined as the angle between the straight line of calibration graph of glucose, and the OX axis (Fig.29). Equation [4] applies to 1:5 diluted solutions of human semen in distilled water, within the

concentration of glucose, which was deducted scale calibration (10 ± to 140 ± 1mg/100mL 1mg/100mL). From equations [4] and [5] follows:

ctg u =174.7915 **[6]**

From equations [5] and [6], we can determine glucose x according to the numerical value of relative absorption of UV = VIS GUVP at 267nm y:

x = 174.7915 y **[7]**

Sample	RP, SP, I (%)	Numeric Abs value for GUVP (Abs value ± SD)	Glucose concentration (mg/100 mL)
1	72, 0, 28	0.32832±0.01429	57.3±2.7
2	60, 9, 31	0.25339±0.01147	44.2±2.3
3	61, 10, 29	0.25911±0.01236	45.2±2.4
4	54, 22, 24	0.26712±0.01161	46.6±2.3
5	59, 11, 30	0.23795±0.01212	41.5±2.4
6	63, 21, 16	0.27742±0.01326	48.5±2.6
7	64, 15, 21	0.27227±0.01291	47.5±2.5
8	52, 27, 21	0.25968±0.01257	45.3±2.4
9	57,16,27	0.23966±0.01269	41.8±2.5
10	59, 11, 30	0.28771±0.01007	50.2±2
11	70, 5, 25	0.30831±0.01402	53.9±2.7
12	66, 8, 26	0.27914±0.01345	48.8±2.7
13	71, 9, 20	0.32147±0.01421	56.2±2.7
14	60, 13, 27	0.23796±0.01367	41.6±2.7
15	53, 29, 18	0.26027±0.01215	45.5±2.4
16	57, 14, 29	0.27914±0.01238	48.8±2.4
17	56, 20, 24	0.27171±0.01326	47.5±2.7
18	57, 18, 25	0.24941±0.01163	43.6±2.3
19	50, 21, 29	0.24253±0.01369	42.4±2.7
20	59, 6, 35	0.29574±0.01146	51.7±2.3
21	58, 11, 31	0.28201±0.01451	49.3±2.8
22	64, 26, 10	0.27229±0.01254	47.6±2.4
23	69, 8, 23	0.27286±0.01362	47.7±2.8
24	67, 2, 31	0.29288±0.01268	51.2±2.5
25	63, 14, 23	0.26313±0.01436	46±2.8
26	54, 11, 35	0.25284±0.01166	44.2±2.3
27	51, 27, 22	0.23969±0.01364	41.9±2.6
28	69, 7, 24	0.30032±0.01329	52.5±2.6
29	62, 6, 32	0.26944±0.01037	47.1±2.1
30	51, 17, 32	0.22657±0.01114	39.6±2.2

Tab.2 Glucose concentration (column 4) in human semen parameters (RP, SP, S) (column 2) obtained for healthy samples (column 1). GUVP Abs peak value relative to 267nm is shown (column 3).

Equation [7] is significant at $p < 0.05$ level, and results for the total concentration of glucose in human sperm, are in the common units for this type of study, mg/100 mL. Calibration graph was determined for natural human sperm diluted 1:5 in distilled water, without any preservatives or chemical additives. We used the method to determine the total concentration GUVP glucose in human sperm samples. We chose a group of 30 healthy and fertile donors.

The results are presented for illustration (Tab.2). We obtained for the average healthy human sperm glucose concentrations 47.17 ± 4.13 mg/100 mL value ($p < 0.05$, $R^2 = 0.9801$) (Tab.2). To validate the method we determined the concentration of glucose in 30 samples collected from healthy donors (Tab.2). The results were compared with those obtained by the biochemical control methods (undisclosed basic table).

Neglecting the second term in equation [3], gives a physical meaning to the equation, for zero glucose concentration, we have a zero relative absorption at 267nm. Error associated with neglect of the second term is ±0.00633mg/100mL, which is insignificant, far below the detection limit of any detection methods currently known for glucose. The method was validated and the results reported [Truța Z et al., 2010].

TEMPORAL VARIATION OF PERCENTAGE OF RAPID AND SLOW PROGRESSIVE CELLS IN GMF

Percentage of fast and slow progressive cells were recorded for haploid male cells during 30 hours exposure in GMF. These experiments were made in vitro at a temperature of 22 ± 0.3^0C. In the first graphic evidence was collected from a very fertile donor, the second graph from a donor with characteristics normozoospermie. All concentration measurements made at a time, have an average relative error of 2%, resulting in the type of method adopted [Makler, 1978].

It is noted that the general trend is that for rapid progressive cells concentration to decrease (Figure 9), and the slow progressive cells concentration to increased for the first period of time, and after a peak todecrease for time the sample is viable. It was suggested that the increase in slow progressive sperm concentration is due to rapid progressive sperm, which are became slowly progressive over time [Truța Z et al., 2006].

In the two cases considered immobile percentage of sperm can be found easily through standardization. Viable nonprogressive sperm virtually are of no interest for in vitro fertilization or artificial insemination, only in special donor cases for which all sperm are viable but nonprogressive, and DNA information is intact.

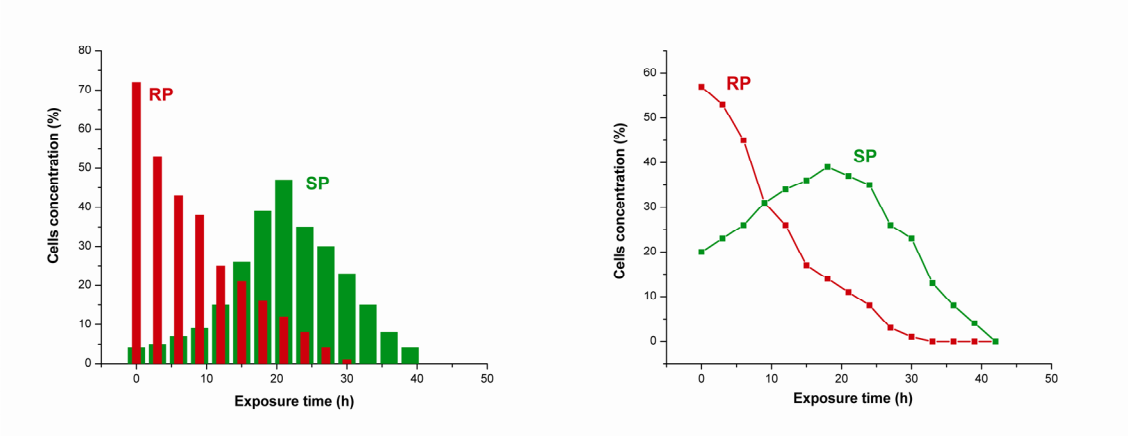

Fig.9 Variation of rapid spermatozoa percentage (red) and slow progressive (green) in a fertile semen sample (72, 4, 24%) of 31 million / mL exposed in GMF (left). Variation in percentage of rapid spermatozoa (red) and slow progressive (green) in a normozoospermic sample (57, 20, 23%) with 26mil/mL exposed in GMF.

ZMF EFFECTS ON HAPLOID CELLS

EFFECTS OF TEMPERATURE ON STRAIGHT LINE VELOCITY (VAP) AND VIABILITY OF HAPLOID MALE CELLS IN ZMF

Sperm are cells which activated motiliatz shortly after semen liquefaction. Velocity of movement of these cells depends on the temperature at which they are exposed. The cryopreservated cells are immobile and above 43^0C they die from hyperthermia.

For in vitro study we worked at room temperature. We made a set of five experiments to determine the effect of temperature on straight line velocity (VAP) of sperm exposed to GMF. Rapid progressive VAP cells present for the 5 temperatures (Fig. 10). The sample was chosen from a fertile patient (72, 0, 28%) 55 million/mL. It is noted that at 17^0C after about 13 hours virtually all cells are immobile. At 24^0C the slow progressive cells even after 30 hours.

When all cells are immobile the sample is no longer considered as viable [WHO, 2001]. Decrease in VAP rates at the five temperatures considered is higher for lower temperatures. Even a difference of only 2^0C average VAP decreasing rate significantly differ. The sample exposed to 24^0C have a 1.06μm/s/h decreasing rate while the average decreasing rate at 22^0C is 1.48μm/s/h.

We present the variation in time of VAP for a normozoospermie semen sample (72, 9, 19%) exposed in ZMF, respectively GMF as control, at 3 different temperatures (Fig. 10). To be mentioned that the average VAP rate decrease is much higher for samples exposed to lower temperatures and for exposures to the same temperature, ZMF exposed sample has a VAP value significantly higher than the average VAP for GMF exposed sample (Fig.

10). Viability also depends on temperature exposure tests, and the value of magnetic flux density. Sample at 24^0C ZMF exposed is no longer viable after 49 hours, while the sample exposed to GMF is not viable after 46 hours. Sample at 21^0C ZMF exposed is no longer viable after 35 hours, while the sample exposed to GMF is not viable after 31 hours. Sample at 17^0C ZMF exposed is no longer viable after 19 hours, while the sample exposed to GMF is not viable after 15 hours.

The evidence presented (Fig. 10) shows that samples are viable when the average VAP is virtually zero. Therefore there is a time when human sperm exposed to a certain temperature no longer has motile progressive cells, but they do have a metabolism, so they still consume glucose (and of course other nutrients). From the intersection of the axis OX with time schedules presented results in which motile sperm are no longer progressive, but are still viable (Fig. 10).

This difference gives an approximate time-dependent temperature exposure: 7h to 24^0C, 5 hours at 21^0C, and with aproximation 3 hours at 17^0C. Note that cryopreservation, freezing very quickly, is quite another phenomenon [Ishikawa et. al, 2007], and the effects recorded and disclosed by us does not apply to these very low temperatures.

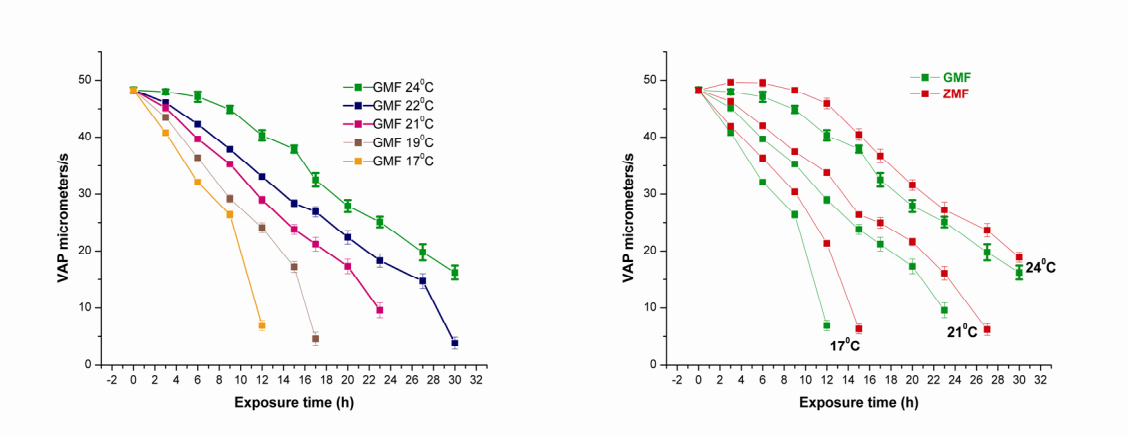

Fig.10 VAP variation of a normozoospermic sample (72, 9, 19%) with 32 million/mL exposed in GMF (left). VAP variation of a normozoospermic sample (72, 9, 19%) with 32 million/mL exposed in ZMF and GMF as control (right), for three different temperatures. Thermostatic error is ± 0.3^{0}C

ZMF INFLUENCES ON VAP OF HUMAN HAPLOID MALE CELLS

Rapidly progressive sperm category is the category responsible for sperm quality and fertility estimates of a patient. This is why we focused mainly on this type of cells. All samples considered were studied to determine the average VAP. Average VAP is maintained higher in samples exposed in ZMF than in samples exposed to GMF throughout the time that cells are motile.

VAP average decrease in ZMF is delayed about 3.5 hours compared to GMFexposure. Even after 30 hours of exposure to this temperature (22 ± 0.3 0C) average VAP still has significant value.

After 30 hours the sample exposed in ZMF has an average VAP value of 19.1 ± 0.86 µm/s while the sample exposed in GMF has an average VAP value of 16.3 ± 1.23µm/s (Fig.11). In all samples of normozoospermic type considered in our study, 22 hours after start of in vitro at 22 0 C (± 0.3 0C), rapidly progressive cells were virtually absent in samples exposed to GMF, while 4% of cells were still rapidly progressive in ZMF exposed samples.

It is noted that the average VAP value is always significantly higher in samples exposed in ZMF compared with samples exposed to GMF, and the results are similar to small differences related to biological samples specific [Truța Z et al., 2005].

Both graphs shown the average VAP, and the deviation error for each time we have performed measurements, show the same type of relative evolution of the sample exposed in ZMF, related to the sample exposed in GMF (Fig.11).

Note that the natural decrease in VAP average time is delayed by about 3.5h in ZMF exposed samples compared with GMF exposed samples.

We suggest that rapid progressive cells percentage increase in the early hours after liquefaction, for ZMF exposed samples, can be explained due to stimulation of cell velocity, and following the passage of cells from slow progressive cells, to the rapid progressive cells.

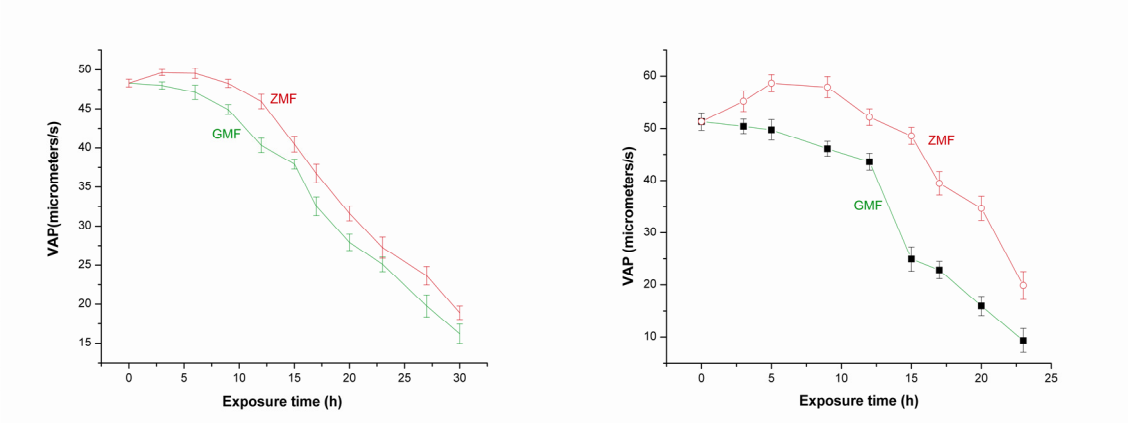

Fig.11 Average VAP variation in time for a normozoospermic very fertile semen sample (72, 0, 28%) with 55mil/ml (left), respectively (51, 17, 32) with 62mil/ml (right) exposed in ZMF and GMF as control. Standard errors of determination are shown for each exposure time considered

ZMF INFLUENCES ON TRAJECTORIES LINEARITY (LIN) OF HUMAN HAPLOID MALE CELLS

Another important parameter is the linearity of cells trajectory. Theoretically keeping other motility parameters constant, a better linearity will induce a better fertility. Sperm category of interest, in terms of estimates of fertility, are rapid progressive cells. Therefore measurements of LIN of cells trajectories have at least 20 randomly chosen sperm cell movement paths, at each point in time when measurements were made.

For convenience we chose sequencial shooting at a frequency of 1Hz, and the sperm coming out of the visual field in less than 4s were not taken into account in determining the LIN of cells trajectories. All determinations of the LIN of cell trajectories in vitro were made at a room temperature of 22 ± 0.3^0C. The same samples exposure (64, 6, 30%) in ZMF and GMF as control parameter, increases LIN of cell trajectories of both samples within first 3 hours of exposure. However ZMF exposed sample cells reach a better trajectory LIN (0.959) than the sample exposed to GMF (0.931) after 3 hours of exposure.

If the second sample set (63, 14, 23%) after 3 hours ZMF exposed sample reaches a peak value of linearity LIN = 0.975 while at the same time after exposure, the GMF has the maximum LIN = 0.924 only.

It is noted that the sample (63, 14, 23%) LIN take higher values exposed in ZMF compared with the same sample exposed to GMF throughout the exposure (Fig. 12).

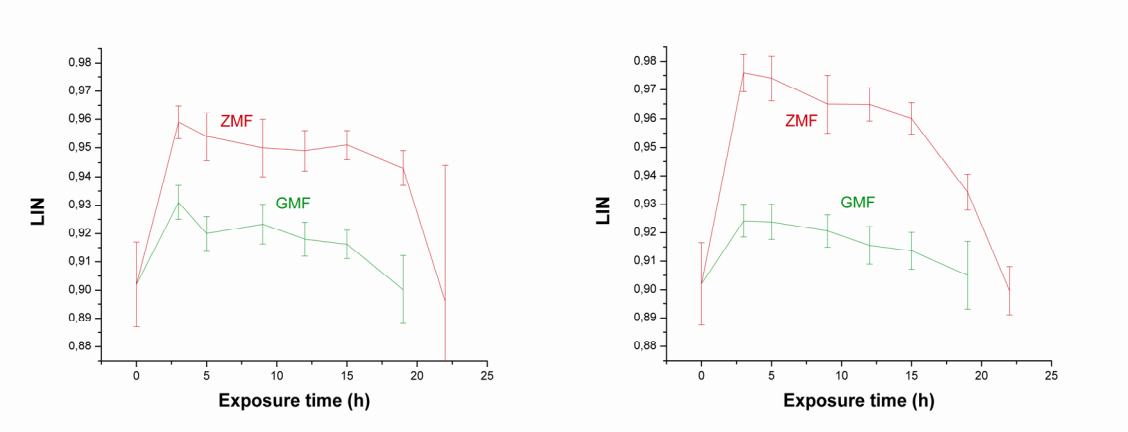

Fig.12 Temporal evolution of the linearity of healty semen sample (64, 6, 30%) (left), respectively (63, 14, 23%) (right), exposed to GMF (green) and ZMF (red)

ZMF INFLUENCES ON AMPLITUDE OF LATERAL HEAD DISPLACEMENT (ALH) OF HUMAN HAPLOID CELLS

Another important parameter of sperm motility is the amplitude of lateral head displacement. To understand this parameter we must look at the cell swimming motion as a system of head, body and tail structurally interconnected.

If to this system we apply the kinetic momentum variation theorem, perpendicular to the direction of movement, it is clear that when the tail moves from one side of the direction of swimming, another structure must balance the kinetic momentum change caused by the tail, with a change of momentum equal and of opposite direction.

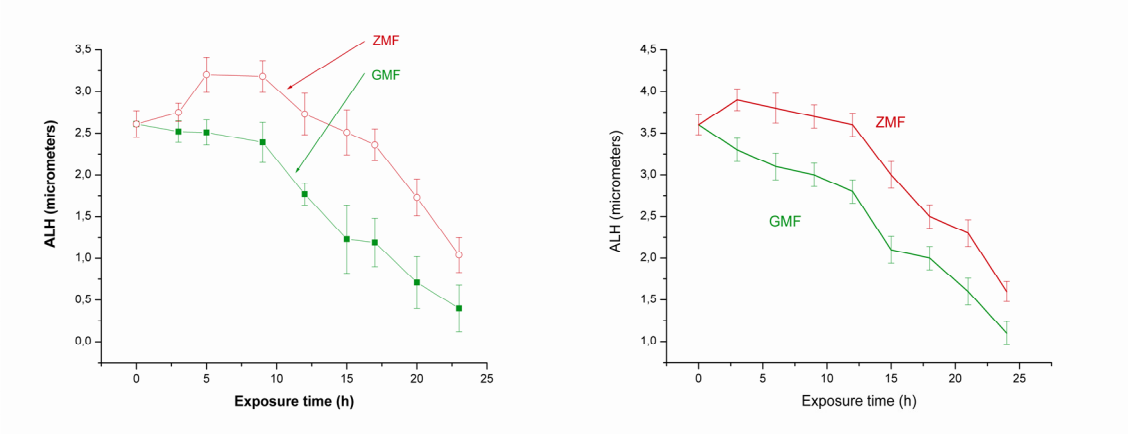

Fig.13 ALH for a healthy sample (64, 6, 30%) (left), respectively (63, 14, 23%) (right) exposed to ZMF and GMF as control. Standard error of deviation is shown for each set of measurements.

Therefore, because the total displacement of the center of gravity of the cell perpendicular to the direction of movement is zero, head and body of the cell will perform a movement to compensate for changes in momentum caused by the beating tail.

As a result the sperm head moves perpendicular to the swim direction as many times each cell will beat, compensating for the kinetic momentum change caused by movement of the tail.

Theoretically because sperm head and body are heavier than the tail, and ALH will be less than the tail beating amplitude. ALH was determined for all samples studied. For this parameter we chose the frequency of 25Hz sequential shooting. This frequency, combined with slow play of cells swimming records, allows the observation and study in detail of the trajectory and head movement.

Note that ALH has a higher value throughout the exposure to ZMF exposed samples compared with samples exposed to GMF (Fig.13). The same type of variation in time of ALH value, based on exposure time reference is found in all samples considered healthy (basic tabel undisclosed).

ZMF INFLUENCES ON TAIL BEAT CROSS FREQUENCY (BCF) OF HUMAN MALE HAPLOID CELLS

Another parameter to consider is BCF. Illustrating the evolution of BCF we present exposure to ZMF, respectively GMF as control, of two healthy samples of counting parameters (64, 6, 30%) and (63,14,23%) (Fig.14). From the graphs presented (Fig.14) results show that the only parameter which decreases at exposure to ZMF compared with GMF, is BCF. All normozospermic samples studied, shows the same type of variation in time, related to BCF motility parameter.

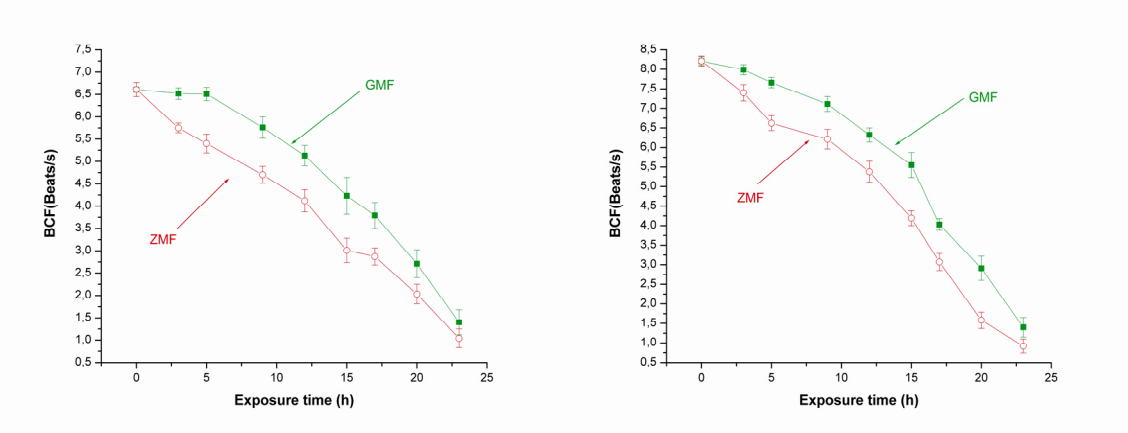

Fig.14 Variation in time of BCF for a healthy sample (64, 6, 30%) (left), respectively (63, 14, 23%) (right), ZMF and GMF exposed as a control. Standard error of deviation is shown for each set of measurements

GLUCOSE CONSUMPTION FOR EXPOSED CELLS IN ZMF AND GMF

Glucose concentration in human sperm samples exposed in ZMF and GMF as control, were determined using the new method developed GUVP [Truța Z et al., 2010]. We used the equation [7] for each set of five measurements and reference absorption considered for each, and all sperm samples analyzed by method specifications [Truța Z et al., 2010]. We present the values of initial and final (after 72h) relative absorbance peaks Abs, extracted using the GUVP method presented, determined at ZMF and GMF exposure as control (Tab. 3).

Sample (#)	GUVP GMF initial (Abs)	GUVP GMF final (Abs)	GUVP ZMF final (Abs)
1	0.2596±0.0125	0.1493±0.0096	0.0715±0.0075
2	0.2396±0.0126	0.1540±0.0099	0.0591±0.0067
3	0.2877±0.0100	0.1727±0.0111	0.0824±0.0096
4	0.3083±0.0140	0.2380±0.0153	0.1229±0.0114
5	0.2791±0.0134	0.2178±0.0140	0.1104±0.0137

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

Tab.3 The initial relative absorbance GUVP Abs (column 2) determined for all samples considered (#). Abs relative absorption value of the final GUVP determined after 72 hours of exposure ZMF (column 4) and the GMF (column 3)

From Tab.3 glucose concentration can be determined for initial and final samples in ZMF and GMF exposed (Tab.4) according to the equation [7].

Sample	Initial glucose concentration (mg/100mL)	Final glucose concentration GMF (mg/100mL)	Final glucose concentration ZMF (mg/100mL)
1	45.3±2.4	26.1±2.8	12.5±2
2	41.8±2.5	26.9±2.1	10.3±1.8
3	50.2±2	30.1±2.3	14.4±2.1
4	53.9±2.7	41.6±2.8	21.4±2.3
5	48.8±2.7	38±2.6	19.3±2.6
6	56.2±2.7	38.8±2.7	19.3±2.7
7	41.6±2.7	25.2±2.1	8.15±1.8
8	45.5±2.4	28.8±2.2	13.5±1.8
9	48.8±2.4	35.9±2.5	20.1±2.6
10	47.5±2.7	35.6±2.6	19.8±2.5
11	43.6±2.3	27.7±2.2	12.5±2.1
12	42.4±2.7	32.6±2.5	18.4±2.4

13	51.7±2.3	35.9±2.6	20.1±2.7
14	49.3±2.8	28.8±2.2	20.3±2.5
15	47.6±2.4	26.1±2.1	11.1±2
16	47.7±2.8	24.2±2.2	6.2±1.7
17	51.2±2.5	26.3±2.3	8.1±1.7
18	46±2.8	30.4±2.3	14.6±2.1
19	44.2±2.3	27.4±2.2	13.3±2.1
20	41.9±2.6	33.4±2.5	18.7±2.3
21	52.5±2.6	29.6±2.2	10.3±2
22	47.1±2.1	28.8±2.2	13.8±2.1
23	39.6±2.2	24.2±2.2	9.2±1.8
24	57.3±2.7	40.5±2.7	20.6±2.3
25	44.2±2.3	35±2.6	18.4±2.6
26	45.2±2.4	26.6±2.1	8.1±1.8
27	46.6±2.3	36.4±2.6	20.6±2.7
28	41.5±2.4	26.3±2.2	10.0±2.1
29	48.5±2.6	33.1±2.5	16.3±2.1
30	47.5±2.5	29.1±2.2	11.1±2.1

Tab.4 Initial glucose concentration (column 2), and samples exposed to 72 hours in GMF (column 3), respectively ZMF (column 4)

Based on the results presented (tab.4) we can calculate the total consumption of glucose in ZMF and GMF as control (Tab.5).

Sample	**RP (%)**	**Glucose total consumption in GMF (mg/100mL)**	**Glucose total consumption in GMF (mg/100mL)**
1	52	19.1±5.2	32.7±4.4
2	57	14.8±4.6	31.4±4.3
3	59	20±4.3	35.7±4.1
4	70	12.2±5.5	32.4±5
5	66	10.7±5.3	29.4±5.3
6	71	17.3±5.4	36.8±5.4
7	60	16.3±4.8	33.4±4.5
8	53	16.6±4.6	31.9±4.2
9	57	12.8±4.9	28.6±5
10	56	11.8±5.3	27.6±5.2
11	57	15.8±4.5	31±4.4
12	50	9.7±5.2	23.9±5.1
13	59	15.7±4.9	31.5±5
14	58	20.4±5	28.9±5.3
15	64	21.4±4.5	36.4±4.4
16	69	23.4±5	41.4±4.5
17	67	24.8±4.8	43±4.2
18	63	15.5±5.1	31.3±4.9

19	54	16.7±4.5	30.8±4.4
20	51	8.4±5.1	23.1±4.9
21	69	22.8±4.8	42.1±4.6
22	62	18.2±4.3	33.2±4.2
23	51	15.3±4.4	30.3±4
24	72	16.7±5.4	36.6±5
25	60	16.7±4.9	25.7±4.9
26	61	18.5±4.5	37±4.2
27	54	10.1±4.9	25.9±5
28	59	15.1±4.6	31.4±4.5
29	63	15.3±5.1	32.1±4.7
30	64	18.3±4.7	36.3±4.6

Tab.5 Total glucose consumption after 72 hours exposure to ZMF (column 4) and in the GMF (column 3), samples of healthy cells, with rapid progressive RP cells concentration (column 2) for all samples considered

PARAMETERS OF STUDY CORRELATION

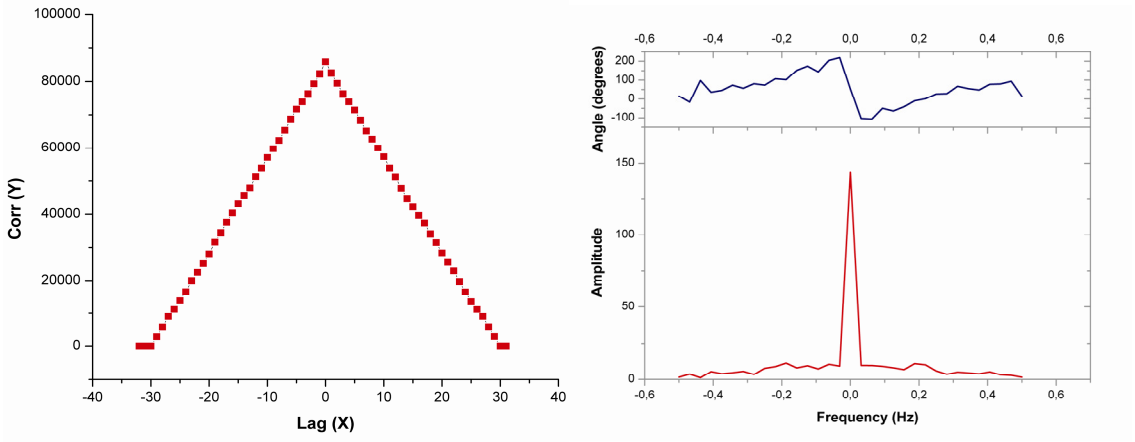

Fig. 15 Correlation between initial RP and initial glucose for all the healthy samples (left). FFT shows an absolute value (amplitude) significant correlation(right).

The results presented suggest a correlation between the percentage of cells initially rapidly progressive RP, and glucose in type normozoospermie samples (Tab.4, Tab.5). To analyze this correlation we used mathematical analysis (Origin 6.1) and Fourier Transform (FFT). Results are presented as mathematical correlation (Fig.15), and as FFT correlation (Fig.15). Healthy samples, samples with higher initial glucose concentration have a higher concentration of rapid progressive cells (Tab.4, Tab.5, Fig.15). If we use Fourier Transform (FFT) with two columns of values, representing the initial concentration of glucose and RP

sperm concentration in healthy sperm samples, the correlation amplitude has the value 143.42 (Fig.15). Also, the final average concentration of glucose in healthy samples exposed in ZMF and GMF as control can be calculated (Tab.4). From the results presented (Tab5) average glucose concentration in human sperm normozoospermic samples can be calculated:

Initial average concentration of glucose = 41.17 ± 4.38mg/100mL [8]
Final average concentration of glucose in ZMF = 14.73 ± 4.74mg/100mL [9]
Final average concentration of glucose in GMF = 31.02 ± 5.0 mg/100 mL [10]

Consumption of glucose for each sample separately, shows that one and the same sample exposed to ZMF consumed significantly more glucose than exposed to GMF. This is checked for each sperm sample in hand, and without exception all samples (Tab.5).

Another correlation of interest is the correlation between RP and the total consumption of initial glucose evidence presented in ZMF and GMF as control.

The results show a good correlation between the initial RP of healthy sample and total consumption of glucose in GMF, and almost doubled between the initial RP of healthy sample and total consumption of glucose in ZMF (Fig.16).

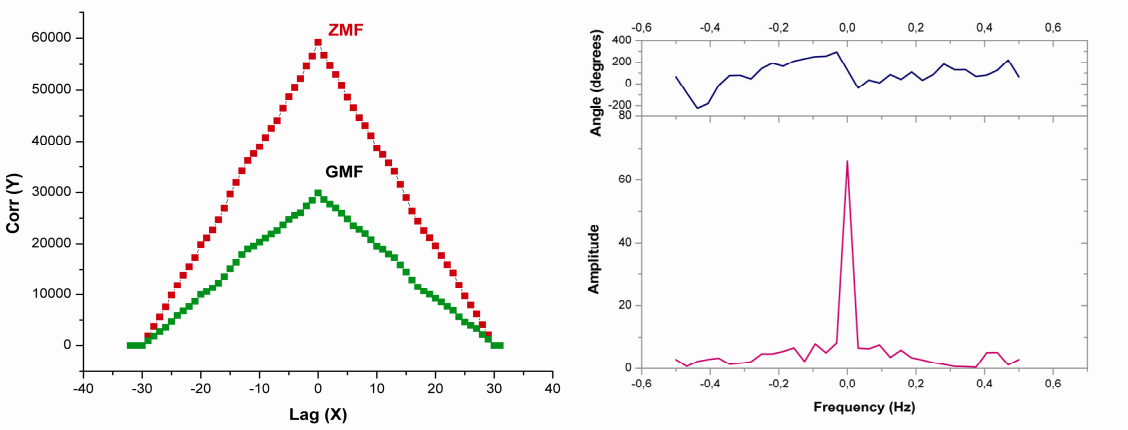

Fig. 16 Correlation between initial RP and total glucose consumption for a healthy sample (63, 14, 23%) exposed in ZMF and GMF considered as control. FFT shows an absolute value (amplitude) corresponding to a good correlation

Mention that the correlation between initial RP and healthy samples glucose consumption is good only for normozoospermic semen samples, where most cells are rapid progressive.

One of the most important correlation for our study is the correlation between ALH and VAP of the same samples. We present the correlation mentioned for a normozoospermic semen sample (63, 14, 23%) exposed to GMF, respectively ZMF (Fig.17). Correlation between ALH and VAP of the same sample exposed to GMF, gives a FFT amplitude of 34.02 (fig.17). Correlation between ALH and VAP of the same sample exposed to ZMF, gives a FFT amplitude of 38.16 (fig.17). It should be noted that the value of Fourier Transform of the correlation amplitude for the same sample exposed to GMF, respectively ZMF between ALH and VAP, is closed.

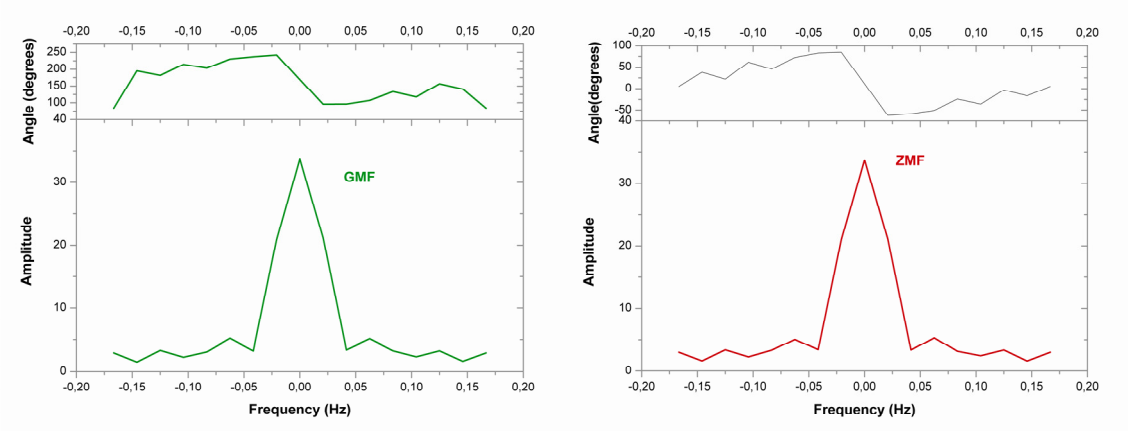

Fig.17 Correlation of ALH with VAP for a healthy sample (63, 14, 23%) by FFT amplitude for the sample exposed to GMF, and same sample exposed to ZMF.

DISCUSSION

Radio frequency electromagnetic radiation (100 kHz - 300 GHz) have negative influences on male reproductive cells, and on their generation (spermatogenesis).

EMF magnetic fields of low frequency (ELF) of pulsating square shape stimulates human male haploid cell motility. Magnetic fields produced by power lines does not significantly influence human male haploid cells, although reports show that they increase the risk of breast cancer and risk of leukemia. Reports that measure this influence are show inconsistent and insignificant results.

Human sperm motility and velocity in vitro depends on the temperature at which sperm are exposed samples (Fig. 10). Human sperm motility and velocity decreases with temperature at room temperature. Albumin has a pronounced UV absorption, but it does not affect extraction of glucose UV-VIS peak at 267nm (Fig.6).

The new method GUVP for glucose consumption, or the concentration of glucose in human sperm samples by quantitative spectrophotometric analysis, give good values, consistent with literature, with acceptable errors (less than 4%) [Truța Z et al., 2010].

Measurement of glucose concentration in human semen samples was done in the past 100 years, but the results reported are rare and differ from 39.50mg/100mL [Ighinaduwa P et al., 2007] to 71mg/100mL [Montagnon D et al. 1982]. Other authors report an average concentration of glucose in human sperm samples 25.8mg/100mL [Tomaszewski L et al. , 1992], or even less, 18.3mg/100mL [Hirsch IH et al., 1991]. A stimulant of sperm has been proposed with 102mg/100mL glucose concentration [Mukai C et al., 2005].The newest reports so far show a concentration of glucose in human healthy sperm of 47.17 ± 4.13mg/100mL [Truța Z et al., 2010]. We excluded from the analysis of comparative results reports before 1980 as not reliable.

Initial concentration of glucose in samples of sperm and the total number of cells in human sperm samples are not correlated (Tab.2). Remember that glucose is the product of human seminal fluid seminal bladder and sperm are produced in the testis, two totally different places of the human body.

Rapid progressive concentration of cells, cells that give estimates of male fertility in human sperm samples, decrease with time and reaches zero after about 30 hours or at room temperature 22 ± 0.30C (Figure 9).

VAP increases motility parameter for samples exposed to ZMF, in the first hours after liquefaction, while VAP decreases for samples exposed in GMF for the first hours after liquefaction at room temperature 22 ± 0.3 0C (Fig.11).

LIN motility parameter increases greater for samples exposed to ZMF, in the first hours after liquefaction, comparing to samples exposed to GMF (Fig.12). Each, and all normoyoospermic semen samples have a better linearity in ZMF compared to exposure in GMF.

Correlation between ALH and VAP (Fig.13) for each sample considered ZMF and GMF exposed (Table undisclosed), shows that to achieve higher speeds sperm must use wider tail beat, and therefore BCF will decrease, and ALH will increase.

BCF motility parameter decreases more pronounced for samples exposed to ZMF, in the first hours after liquefaction in comparison with samples exposed to GMF (Fig.14).

The results show that after 72 hours of exposure ZMF average glucose consumption is 26.97 ± 4.9mg/100mL while after exposure to the same period the average consumption of glucose in GMF is 10.15 ± 4.1mg/100mL [9, 10].

These results were compared with previous published results [Barak Y et al., 1998, Mahaven MM et al., 1997, Williams C et al., 2001, Miki K et al. 2004, Truța Z et al., 2011] for confirm the importance of glucose as a major bioenergetic substrate for the sperm motility.

After 5 hours of exposure in human sperm samples ZMF increase their concentration of RP cells on average 10.23% (66.43% from 60.26%, $P < 0.05$) compared with a decrease in the concentration of cells with 3.31% for proper RP samples exposed to GMF as control (58.26% from 60.26%, $P < 0.05$) (tab.6).

Scale calibration we determined (Fig.8, equation [7]) can be used for quantitative spectrophotometric analysis with acceptable errors (less than 0.0059 Abs, ie below 0.6 mg/100 mL) at dilution of 1:5.

We suggest that much lower dilutions than those in which we determined the calibration scale can not be used because changes in concentrations of chemical components are not linear with relative absorption intensity. It is known that relative absorption intensity for undiluted samples varies with the concentration after a saturation curve, where obviously a linear fitting can not be used

GEOMAGNETIC ACTIVITY CONSIDERATIONS

Since during our experiments Ap index had values below 15 (the minor magnetic storm), experiments were made during a quiet geomagnetic activity (Fig.18). This shows that the experiments have not been influenced by magnetic storms. Time when the experiments were performed is defined in the chart presented between arrows (Fig.18).

Also all other types of electromagnetic usual radiation of EMF, ELF has not been screened, they are the same for the evidence presented in ZMF and GMF exposed samples. We conclude that the effects recorded were caused by lack of GMF, and not for other factors.

Geomagnetic activity (GMA) measures the natural fluctuations of Earth's magnetic field and is measured with Ap index and K index of GMA. These indices were taken from reports of the National Geophysical Data Center of USA [USAF Estimated Press Index, 2010].

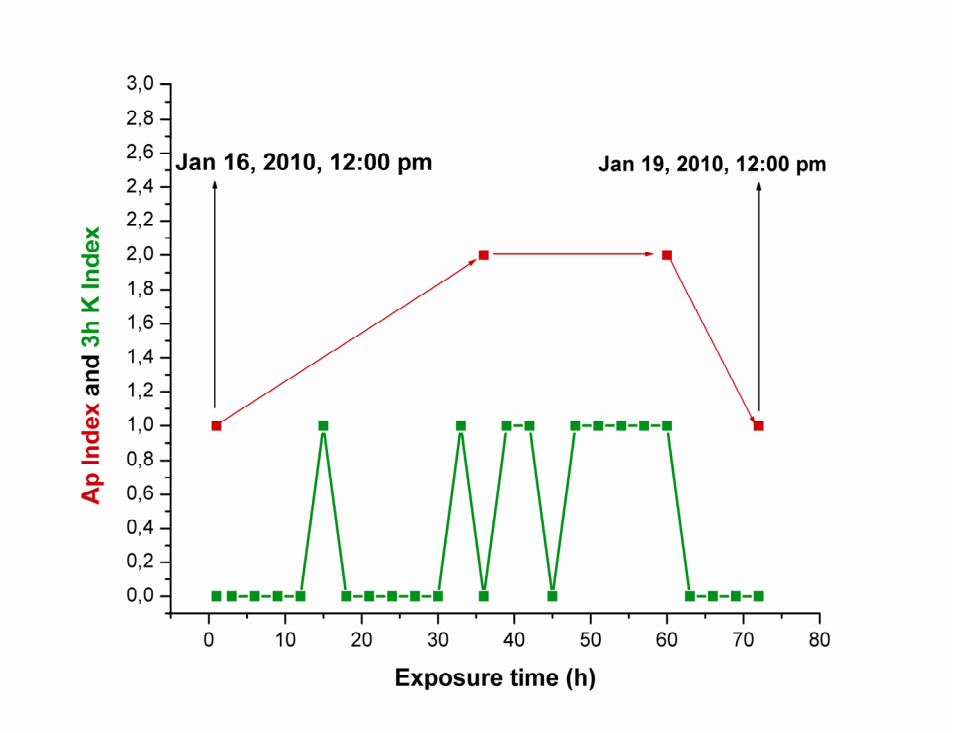

Fig.18 Geomagnetic activity during the period in which human sperm samples were exposed to ZMF and GMF

CONCLUSIONS

ZMF stimulates human sperm velocity VCL and VAP in vitro, compared with GMF (Fig.11).

ZMF stimulates human sperm LIN of cell trajectories in vitro, compared with GMF (Fig.12).

ZMF stimulates human sperm ALH in vitro, compared with GMF (Fig.13).

Exposure to ZMF for normozoospermic human semen samples it reduces their BCF compared to GMF exposure (Fig.14).

The correlation between glucose consumption of the evidence presented in ZMF and GMF proportion of initial RP cells, is suggesting that consumption of of glucose of healthy human sperm is used mostly for their motility (Tab.5, Fig.16). Similar results were reported for other mammals [Mukai C et al., 2004].

The method developed allows non-destructive determination of glucose concentration in human semen samples and human seminal fluid. Our results show that assesement of concentration of glucose in human sperm, based on UV-VIS spectrophotometry, is possible

with the same degree of sensitivity as the current technology, and it gives more flexibility in specific areas of research.

The concentration of glucose in seminal fluid is an important bioenergetic parameter, and we recommend GUVP method developed by us to study human sperm metabolism.

After 5 hours of exposure in ZMF human normozoospermic sperm motility was significantly increased (Fig.11, Fig.12). These results suggest that a simple in vitro ZMF exposure may increase the estimates of human sperm fertility.

We suggest that the effect of stimulating human sperm motility of ZMF compared to GMF, is due to increased glucose consumption in ZMF, compared with GMF, which increases ATP synthesis of biological fuel. Increasing the ATP concentration in semen significantly stimulates human sperm motility.

Analysis summary of the results shows that GMF induces significant disorder at the molecular level for human male haploid cells, and compensating the GMF, getting ZMF, we can significantly minimize the inhibitory effect of GMF, on motility parameters of human male fertility.

REFERENCES

1. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P., Cilia and Flagella Are Motile Structures Built from Microtubules and Dyneins, Molecular motors, Molecular Biology of the Cell, (2002)
2. Asashima M, Shimada K, Pfeiffer CJ. Magnetic shielding induces early developmental abnormalities in the newt, Cynops pyrrhogaster. Bioelectromagnetics, 12(4): 215-224 (1991)
3. Barak Y., Goldman S., Gonen Y., Nevo Z., Bartoov B., Kogowski A., Does glucose affect fertilization, development and pregnancy rates of human in-vitro fertilized oocytes?, Hum. Reprod., 13, 4203-4211, (1998)
4. Cao W., Haig-Ladewig L., Gerton G. L., Moss Stuart B., Adenylate Kinases 1 and 2 Are Part of the Accessory Structures in the Mouse Sperm Flagellum, Biol. Reprod.,
5. Courtillot V, Le mouel JL. Time variations of the Earth magnetic field: From daily to secular. Annual review of Earth and Planetary Science, 16:435-439 (1988)

6. De Vita R, Cavalo D, Raganella L, Eleuteri P, Grollino MG, Calugi A. Effects of 50 Hz magnetic fields on mouse spermatogenesis monitored by flow cytometric Analysis. Bioelectromagnetics 16:330-334 (1995)
7. Du J., Tao J., Kleinhans F.W., Mazur P., Critser J.K. Water volume and osmotic behaviour of mouse spermatozoa determined by electron paramagnetic resonance. Journal of Reproduction and Fertility 101: 37-42 (1994)
8. Emura R, Ashida N, Higashi T, Takeuchi T. Orientation of bull sperms in static magnetic fields. Bioelectromagnetics, 22(1): 60-65 (2001)
9. Fletcher DA si Theriot JA. An introduction to cell motility for the physical scientist. Phys Biol. 2004 Jun;1(1-2):T1-10 (2004)
10. Gailliot, M.T., Baumeister, R.F., DeWall, C.N., Maner, J.K., Plant, E.A., Tice, D.M., Brewer, L.E., & Schmeichel, B.J. "Self-Control relies on glucose as a limited energy source: Willpower is more than a metaphor." Journal of Personality and Social Psychology 92: 325-336 (2007)
11. Gajewski E, Steckler D, Goldberg R. "Thermodynamics of the hydrolysis of adenosine 5'-triphosphate to adenosine 5'-diphosphate". J Biol Chem 261 (27): 12733–7. PMID 3528161 (1986)
12. Ginsburg KA, Moghissi KS, Abel EL. Computer-assisted sperm analysis. Sampling errors and reproductibility, Journal of Andrology, (9), p.82-90, (1988)
13. Herndon J.M."Nuclear georeactor origin of oceanic basalt 3He/4He, evidence, and implications"Proceedings of the National Academy of Sciences 100 (6): 3047-3050 (1991)
14. Hiroyuki T, Iijima S, Nakanishi Y, Kamiguchi Y, Asaka A. No induction of chromosomes aberrations in human spermatozoa exposed to extremely low frequency electromagnetic fields.Mutation Research, 414:31-35 (1998)
15. Hirsch IH, Jeyendran RS, Sendor J, Rosecrans RR, Staas WE.Biochemical analysis of electrosjaculates in spinal cord injured men: comparison to normal ejaculates. J. Urology.154: 73-76 (1991)
16. Ighinaduwa P, Ighinaduwa B, The Predictive Value of Glucose-fructose Ratio in Seminal Plasma, World Applied Sciences Journal, 2, 602- 605, (2007)
17. Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Suntucci R, Tettamanti E, Colonna R. 2007. A preliminary

study of oscillating electromagnetic field effects on human spermatozoon motility. Bioelectromagnetics 28:72-75 (2007)

18. Ishikawa H. and Kaneko S. Human Sperm Cryopreservation —Theory and Clinical Application. J.Mamm.Ova.Res., Vol. 24, 14-17, (2007)
19. Ishijima S., Digital image analysis of flagellar beating and microtubule sliding of activated and hiper activated sperm flagela, 10 th. International symposium, Madrid, 09-3, 43 (2006)
20. Jequier A.M., Crish J.P., Semen analysis: a practical guide, Blackwell Scientific Publications, Oxford, (1986)
21. Kirschner KN, Woods RJ "Solvent interactions determine carbohydrate conformation". Proc. National Acad. Sci. USA. 98 (19): 10541-10545 (2001)
22. Kowalczuk CI.Biological Effects of Exposure to Non-ionizing Electromagnetic Fields and Radiation. I. Static Electric and Magnetic Fields (NRPB-R238). Natl Radiat Protec Board, Chilton, (1991)
23. Kubota H, Ogiwara Y, Matsuzaki K, Photo-Induced Formation of Peroxide in Saccharides and Related Compounds, Polymer Journal, 8, 557-563, (1976)
24. Lodish, H, Berk A, Matsudaira P, Kaiser CA, Krieger M, Scott MP, Zipursky SL, Darnell J. Molecular Cell Biology, 5th, New York: WH Freeman. (2004)
25. Mahaven M.M., Miller M.M., Moutos D.M., Absence of glucose decreases human fertilization and sperm movement characteristics in vitro, Hum. Reprod., 12, 119-123, (1997)
26. Makler A. A new multiple exposure photography method for objective human spermatozoa motility determination. Fertil. Steril. 30:192–199 (1978)
27. Makler A, Tatcher M, Vilensky A, Brandes JM. Factors affecting sperm motility. III. Influence of visible light and other electromagnetic radiations on human sperm velocity and survival. Fertility and Sterility 33:439-444 (1980)
28. Makler A, Murrilo O, Huszar G, Tarlatzis B, DeCherney A, Naftolin F. Improved techniques for collecting motile spermatozoa from human semen. Intrenational Journal of Andrology. Vol. 7 (1) 61-70 (1984)
29. Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, Perreault SD, Eddy EM, O'Brien DA. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A 101:16501–6 (2004)

30. Miller G. Exposure guidelines for magnetic fields. Amer Indust Hygiene Assoc J 48:957-968 (1987)
31. Montagnon D, Clavert A, Cranz C. Fructose, proteins and coagulation in human seminal plasma, Andrologia, 14, 434- 439, (1982)
32. Moulder JE, Foster KR. Biological effects of power-frequency fields as they relate to carcinogenesis. Proc Soc Exp Biol Med 209:309-324 (1995)
33. Mukai C, and Okuno M. Glycolysis Plays a Major Role for ATP Supplementation in Mouse Sperm Flagellar Movement. Biol. Reproduction 71, Vol.2. pp 540-547 (2004)
34. Narra VR, Howell RW, Goddu SM, Rao DV. Effects of a 1.5-Tesla static magnetic field on spermatogenesis and embryogenesis in mice. Invest Radiol, 31(9): 586-590 (1996)
35. NIOSH Health Hazard Evaluation Report: Alumax of South Carolina. Cent Disease Control Prevention, NIOSH, (1994)
36. Owen D.H., Katz D.F., A review of the physical and chemical properties of human semen and the formulation of a semen stimulant. Journal of Andrology, Vol. 26 (4):459-469 (2005)
37. Ramadan L.A., Adel R.A. Abd-Allah, Hamdy A.A. Aly, Aisha A. Saad-El-Din, Testicular toxicity of magnetic field exposure Nad prophylactic role of coenzyme Q 10 and L-carnitine in mice. Pharmacological research, 46 (2002)
38. Romac P, Grubišić T, Čulić O, Cvitković P, Flogel M. Sperm motility and kinetics of dynein ATPase in astheno- and normozoospermic samples after stimulation with adenosine and its analogues. Human Reproduction, Vol. 9. No.8. pp 1474-1478 (1994)
39. Rossato M, La Sala G B, Balasini M, Taricco F, Galeazzi C, Ferlin A, Foresta C. 1999. Sperm treatment with extracellular ATP increases fertilization rates in in-vitro fertilization for male factor infertility. Human Reproduction 14, Vol.3. pp 694-697
40. Stuchly MA Human exposure to static and time-varying magnetic fields. Health Phys 51:215-225 (1986)
41. Supino R, Bottone MG, Pellicciari C, Caserini C, Bottiroli G, Belleri M, Veicsteinas A.Sinusoidal 50 Hz magnetic fields do not affect structural morphology and proliferation of human cells in vitro., Histol Histopathol 16: 719-26 (2001)

42. Tablado L, Francisco Pérez-Sánchez F, Núñez J, Núñez M , Soler C. Effects of exposure to static magnetic fields on the morphology and morphometry of mouse epididymal sperm. Bioelectromagnetics. Vol. 19 (6) 377–383 (1998)
43. Takeuchi T, Nakaoka Y, Emura R, Higashi T. Diamagnetic Orientation of Bull Sperms and Related Materials in Static Magnetic Fields. Journal of the Physical Society of Japan 71, Vol.1. pp 362-368 (2002)
44. Tomaszewski L, Konarska L, Janczewski Z, Skarzynska E, Lebioda K, Hryckiewicz L. Fructosamine in human and bovine semen, Life Sci., 50 (3), 181, (1992)
45. Truța Z., Neamțu Silvia, Morariu V. Vasile, The influence of zero magnetic field on in vitro human spermatozoa cells behavior, Advanced biomaterials and biophysical techniques, may 26-28,2005, 8thNational Biophysics Conference (2005)
46. Truța Z., Neamțu Silvia, Morariu V. Vasile, Spermatozoa aging is slowed down in ZMF, Isotopic and molecular processes, Fourth PIM Conference, Cluj-Napoca, sept.22-24,(2005)
47. Truța Z, Neamțu S, Morariu VV. Zero magnetic field influence on in vitro human spermatozoa cells behavior. Rom.J.Biophysics 15:73-77 (2005)
48. Truța Z, Lerințiu S, Gârlovanu M, Morariu VV. Zero magnetic field influence on male reproductive cells progressive motility distribution. Sudia Universitatis Babes-Bolyai.Physica LI 1:39-43 (2006)
49. Truța Z., Gârlovanu Marinela, Lerințiu Stela, Micu R. A new methos for human semen glucose concentration evaluation, Romanian Biotechnological Letters, Vol.15 (6) 5764-7572 (2010) or on line at:http://www.rombio.eu/rbl6vol15/cuprins.html
50. Truța Z., Truța M., Micu R. Zero magnetic field influence on human spermatozoa glucose consumption, Accepted for publication by Roumanian Biotechnology Letters, Vol.16 (5) article nr.313 (2011)
51. USAF Estimated Ap index available from: http://sec.noaa.gov/Data/index.html#indices
52. Wenlei C. Adenylate Kinases 1 and 2 Are Part of the Accessory Structures in the Mouse Sperm Flagellum, Biol. Reprod., Jun 21; p.1679-1685, (2006)
53. Williams A.C., Ford W. C., The role of glucose in supporting motility and capacitation in human spermatozoa, J. Androl., 22, 680-695, (2001)

54. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interactions. 3rd ed. Cambridge, United Kingdom: Cambridge University Press; 1999. Biology of Reproduction 65, 1224-1231 (2001)
55. WHO. Electromagnetic fields and public health (2006)
56. Yang C, Owen HA, Yang P. Dimeric heat shock protein 40 binds radial spokes for generating coupled power strokes and recovery strokes of 9 + 2 flagella. The Journal of Cell Biology, Vol. 180, No. 2, 403-415 (2008)
57. Ying J, Grishaev A, Latham MP, Pardi A, Bax A, Glover PM, Bowtell R. Magnetic field induced residual dipolar couplings of imino groups in nucleic acids from measurements at a single magnetic field. J Biomol NMR 39(2):91-6 (2007)