Disturbances of electrodynamic activity affect abortion in animals

M Nedbalova¹, A Jandova², and A Dohnalova¹

¹Institute of Physiology, First Faculty of Medicine, Charles University, Albertov 5, 128 00 Prague 2, Czech Republic
²Institute of Photonics and Electronics, v.v.i., Academy of Sciences of the Czech Republic, Chaberska 57, 182 51 Prague 8, Czech Republic

E-mail: martina.nedbalova@lf1.cuni.cz

Abstract. A specific kind of intracellular organelles, the mitochondria, is the place of metabolic energy production by oxidative mechanism. We used cell-mediated immunity method for verification of the energy metabolism (ATP production). The antigen (immunological functional RNA) was obtained from blood of inbred laboratory mice strain C3H/H2K, infected with the lactate dehydrogenase elevating virus (LDV) and prepared by the high pressure gel chromatography (HPGC). We have studied the immunological adaptability of LDV viral antigen in 62 pigs (12 parents and 50 piglings). Exitus of piglings was in case of positive immunological response on LDV. The statement results from a comparison of the relative frequency of an incidence of identical findings in male piglets and sows and from identical findings in female piglets and pigs. The efficient elaboration and utilization of energy in cell may be damaged by the changes of energy production systems and also by long-term parasitary depletion of ATP energy. Biological activity is based not only on biochemical but also on biophysical mechanisms. Biophysical processes are also involved in the transfer of information and its processing for making decisions and providing control, which are important parts of biological activity. These experimental results were used for the same study in human.

1. Introduction
Research of infection by mice LDH virus (Riley virus) was going on in years 1965-1994 on Institute of Physiology of Universal Medicine Faculty in Prague. As a model was used mice strain C3H/H2K, rats, sewer rats, rabbits and dogs [1,2,3,4]. The model was extended by farm animals (cattle and pigs) [5,6]. The mode of the LDV transfer from mice to other animals was studied. The infection is transmitted from mice to mice by means of saliva, sparmatic fluid and urine [7]. Statistical significant differences in cell-mediated immunity (CMI) answer to antigen obtained from blood of inbred laboratory mice strain C3H/H2K, infected with the LDV were discovered in cows, bulls and their calves [8].

The CMI is important for the immunity of the organism to some viruses, moulds, and/or bacteria living inside cells and tumours, and it is also involved in a potential refusal of the transplant. The CMI was tested using the method of leukocyte adherence inhibition (LAI). An antigen as obtained from blood of inbred laboratory mice strain C3H/H2K infected with the LDV and prepared by the high pressure gel chromatography (HPGC) was used in the test. Pig males, sows and their progeny were used as a model, and their immunity response to the antigen applied was studied (immunologically active LDH-elevating virus RNA). For its survival, a cellular energy metabolism product, i.e. the ATP as formed prevailingly in mitochondria, is utilized by the given RNA. [9].
2. Materials

Preparation of Nonspecific Antigen – LDH Virus Antigen (Immunologically Functional Fraction from the Inbred Strain Mice Blood)

The immunologically functional fraction was obtained from the serum of inbred laboratory mice strains, e.g., C3H/H2K, infected with the virus (LDH) enhancing the lactate dehydrogenase isoenzyme (NAD 1.1.1.27 Oxidoreductase). Plasma obtained from blood treated with heparin was subjected to at least doubled centrifugation for 20-30 minutes at 3-3.5 \times 10^3 \text{ g}, and the supernatant taken was clarified for 1-2 minutes at 9-11 \times 10^3 \text{ g}. The supernatant was subjected to further centrifugation for 50-70 minutes at 50-100 \times 10^3 \text{ g}, and the sediment was suspended in buffer with pH of 7-7.4 consisting of tris-(hydroxymethyl)-aminomethane-NaCl [Tris-NaCl] and the disodium salt of ethylenediaminetetraacetic acid (EDTA) in a minimum volume of at most 1 ml. Equilibrium centrifugation in a saccharose density gradient separated the constituents, and the area corresponding to 32–38 % saccharose with the maximum of 35 % saccharose. This fraction was taken off and, after dilution with the above-mentioned buffer at the volume ratio of 1:0.5 to 1.5, was subjected to centrifugation at 50-100 \times 10^3 \text{ g}, for 50 to 70 minutes. Manipulation of blood plasma until the last sediment was obtained was carried out at 2 to 6°C.

The sediment obtained was dissolved in 1.5-3.0 ml of saline solution and used for high pressure gel chromatography. The fractions corresponding to molecular masses of 3 \times 10^5 to 1.5 \times 10^4 were immunologically active. The equilibrium centrifugation was carried out for a period of 180 to 210 minutes at 150 to 180 \times 10^3 \text{ g}. Separon Hema-300 glc®, which is a suspension copolymer of 2–hydroxyethyl-methacrylate with ethylenedimethacrylate with an exclusion limit of the column of 3 \times 10^5, was used as a stationary phase for the high pressure gel chromatography [10]. Gel particles size was 32 to 40 μm. A differential refractometer and UV spectrophotometer with variable wavelength were used for detection. Saline solution was used as a mobile phase and the active fraction is captured at the wavelength of 340 nm [11].

3. Methods

3.1. LAI (Leukocyte Adherence Inhibition test)

Venous blood (8 ml) was taken from the patient and transferred into a test tube with 500 U of heparin, and incubated in a thermostat for 30 minutes at 37°C. A Pasteur pipette was used to transfer the blood rich in white blood cells into a new test tube. The contents were diluted with a saline solution, medium to its original volume and centrifuged at 1500 - 2500 rpm.

The supernatant was poured off and the sediment was layered with 1 ml of distilled water, bubbled through a Pasteur pipette, and lysis of erythrocytes was achieved. Thereafter, dilution to volume with above mentioned solution according to an option was done. Thus, the lysis of erythrocytes was stopped. The contents were centrifuged once again for 10 minutes at 1500 - 2500 rpm. The rinsing solution was poured off, the sediment of white blood cells was diluted with a saline solution and diluted again so as to make available 4 \times 10^6 cells per 1 ml for the test, as counted in the Bürker chamber.

Suspension with the white blood cells prepared according to the method described above was mixed with antigen at a ratio of 1 part of antigen to 4 parts of white blood cell float. 1 ml of the suspension with T lymphocytes sedimented in the test tubes from green Sial glass. After 60 minutes of sedimentation-adhesion process at 37°C number m of non-adherent cells was counted.

Cells, which didn’t meet the offered antigen, adhere steady to the test tube surface. The sensibilized cells don’t adhere.

3.2. The evaluation of LAI

The results of leukocyte adherence inhibition are expressed as a relative number \( N \) of non-adherent cells: \( N = \frac{m}{M} \times 100 \) (in %) or index of positivity: \( \text{IP} = \frac{N}{33} \)

\( M \) is the number of cells in suspension before sedimentation-adhesion process.
$m$ is the number of non-adherent cells after 60 minutes incubation. 
33 is percent of non-adherent cells obtained continuously from healthy blood donors (1965-1995).
All examinations were practiced as a clinical-blind experiment (without the primary diagnosis) [12].

4. Results
The immunological response to LDV antigen was tested in pig, sow and their piglets. The results of examinations were expressed as index positivity (IP) values. The dependence of IP values in piglets on IP values in other sex parents was studied.

Table 1. Dependence of finding in pig male resp. female descendant on the finding in father resp. mother.

| Father positive | Descendant | | | | | |
|---|---|---|---|---|---|---|---|
| | | male | | female | | descendant |
| | Mother | positive | negative | total | positive | negative | total | total |
| positive | 12 | 1 | 13 | 21 | 0 | 21 | 34 |
| negative | 1 | 17 | 18 | 17 | 4 | 21 | 39 |
| total | 13 | 18 | 31 | 38 | 4 | 42 | 73 |

The positive pig father had 91% of positive piglet daughters by transsexual transfer.

Table 2. Dependence of finding in pig male resp. female descendant on the finding in father resp. mother.

| Father positive | Descendant | | | | | |
|---|---|---|---|---|---|---|---|
| | | male | | female | | descendant |
| | Mother | positive | negative | total | positive | negative | total | total |
| positive | 12 | 1 | 13 | 21 | 0 | 21 | 34 |
| negative | 10 | 2 | 12 | 1 | 11 | 12 | 24 |
| total | 22 | 3 | 25 | 22 | 11 | 33 | 58 |

The positive sow had 88% of positive piglet sons by transsexual transfer.

Table 3. Dependence of finding in pig male resp. female descendant on the finding in father resp. mother.

| Father negative | Descendant | | | | | |
|---|---|---|---|---|---|---|---|
| | | male | | female | | descendant |
| | Mother | positive | negative | total | positive | negative | total | total |
| positive | 10 | 2 | 12 | 1 | 11 | 12 | 24 |
| negative | 0 | 20 | 20 | 2 | 23 | 25 | 45 |
| total | 10 | 22 | 32 | 3 | 34 | 37 | 69 |

The negative pig father had 92% of negative piglet daughters by transsexual transfer.
Table 4. Dependence of finding in pig male resp. female descendant on the finding in father resp. mother.

| Mother negative | Descendant | | | | |
|-----------------|------------|-----------------|-------------|----------------|
| | Male | Female | Descendant |
| Father | positive | negative | total | positive | negative | total | total |
| positive | 17 | 21 | 38 | 4 | 25 | 29 | 84 |
| negative | 20 | 23 | 43 | 2 | 25 | 27 | 45 |
| total | 37 | 46 | 84 | 6 | 50 | 56 | 116 |

The negative pig sow had 97% of negative piglet sons by transsexual transfer.

Table 5. Dependence of the finding in pig descendant on parent of the other sex.

| | Father | | | | Mother | |
|-----------------|-----------|-----------------|-------------|----------------|
| | Female | Male | | | Male | Male |
| | positive | negative | positive | negative | positive | negative |
| positive | 38 | 3 | 22 | 1 | 1 |
| negative | 4 | 34 | 3 | 37 |
| X.square | 53.5 | 47.7 |
| significance | P < 0.0001 | P < 0.0001 |

Table 6. Mean values of IP in pig progeny, separately for positive and negative father and mother, and result of the test of mean values in progeny of positive and negative parent.

| Descendant | Father | Significance | Mother | Significance |
|------------|--------|-------------|--------|-------------|
| | positive | negative | t - test | positive | negative | t - test |
| Male | 1.40 | 1.33 | - | 2.28 | 0.76 | - |
| Female | 2.33 | 0.78 | P < 0.0001 | 1.84 | 1.43 | P < 0.0001 |

Finally, the identical finding in immunological response to LDV antigen, obtained from blood of inbred laboratory mice strain C3H/H2K, infected with the lactate dehydrogenase elevating virus, in pig males and piglet daughters was 91%. The identical finding in sows and piglet sons was 94%. The dependence of finding on other sex parent is statistically high significant.

All these results became a base for research in human population.

5. Discussion

In one third of cases an increased content of the LDH-elevating virus RNA was ascertained in serum of experimental animals of a lower body mass (mice, rats/ sewer rats, and dogs). Therefore, an immune response to the LDH virus antigen was studied in animals of a higher body mass (pigs). As known from literature, the LDH virus infection depends on the mice strain, age and gender and exhibits long-term parasitism on the cellular energy metabolism, which was proven specifically in the genealogical analysis of the frequency of LDH-elevating virus RNA incidence in parents and their progeny. The presence of the LDH-elevating virus RNA was studied in piglets (male or female) in dependence on the finding in their father or mother. It is evident from the results obtained that the identical result in the pig male and his piglet daughters was found in 91 %, while in case of a sow and her piglet sons the identical finding was in 94 %. If the increased LDH-elevating virus RNA occurred in both the parents, the positive progeny died without achieving a normal birth mass. If both the male and female organisms were burdened with the presence of a high amount of the LDH-elevating virus RNA (a high IP), the energetic metabolism of the pregnant sow is insufficient and it does not provide the fetus with a sufficient quantity of ATP through the placenta.
The ATP production (oxidative phosphorylation) is catalyzed by the ATP-synthase localized on the inner mitochondrial membrane. Nevertheless, if LDV is a parasite on the oxidative energy production system (i.e. on the mitochondrial production) of infected cells, the process monitored by the nonspecific antigen might be connected with disturbances of this system [13,14].

6. Conclusions
1) We studied a dependence of the LDH-elevating virus RNA incidence in piglets on the finding in a parent of an opposite gender than that of the offspring. A statistically significant 91% coincidence was found in a pig male and his female piglets. In a sow, the finding exhibited a 94% coincidence with that of male piglets.
2) If an increased LDH-elevating virus RNA occurred in both parents, their positive progeny either did not achieve the standard birth mass or they died.
3) The high content of LDH-elevating virus RNA in mitochondria affects the cellular energy metabolism.

7. References
[1] Motyčka K, Jandová A and Pezlarová J 1979 Studium přenosu infekce LDH virem z rodičů na potomstvo u myší kmene H. Sb. lék. 78 176-80
[2] Motyčka K, Jandová A, Herzog P, Boštík J and Štěpánová I 1984 The lactic dehydrogenase virus (LDV) infection of C3H mice increases the number of spleen plaque forming cells (SPFC) above physiological values. Physiol. Bohemoslov. 33 275
[3] Jandová A, Bendl J, Nedbalová M, Trojan S and Vávrová M 1994 Cell mediated immunity induced by the mouse LDH virus in rats of the Wistar strain. Physiol. Res. 43 20
[4] Jandová A, Bendl J, Nedbalová M and Trojan S 1995 The Inhibition Adherence of Leukocytes in Relation to Specific Rabbit Antibody and to RNA Polymerase. Phys. Res. 44 23P
[5] Říha J and Jandová A 1993 A preliminary study of lactate dehydrogenase virus (LDV) activity in breeding bulls. Živočišná výroba 38 583-90
[6] Nedbalová M, Chválová S, Jandová A and Trojan S 1997 Immunological response of CD4 lymphocytes on LDV antigen in pigs and human parents and children. Arch. Physiol. Biochem. 105 240
[7] Riley V 1974 Biological Contaminants and Scientific Misinterpretations. Cancer Res. 34 1752-54
[8] Říha J, Jandová A and Polášek M 1993 The effects of lactate dehydrogenase virus (LDV) on reproductive performance and health of bovine females. Živočišná výroba 38 233-38
[9] Jandová A, Motyčka K, Čoupek J, Sanitrák J, Heyberger K, Laurová L, Mejsnarová B, Novotná J, Pezlarová J, Macholda F, Šimonová J, Dostál C and Kubát Z 1979 "Disease" of the cellular energy system. Sb. lék. 81 321-27
[10] Jandová A, Sanitrák J, Motyčka K, Pezlarová J and Čoupek J 1978 Isolation of immunoreactive components from experimental and human tissues and serums by high performance gel chromatography. J. Chromatogr. 146 253-60
[11] Jandová A, Čoupek J, Sanitrák J, Motyčka K, Mach O and Pekárek J 1989 Method of preparation of immunologically functional fraction from the inbred strain mice blood. Authors Certificate No. 259704, Prague.
[12] Jandová A, Kobílková J, Čoupek J, Vejborn O, Postupa J and Pokorný J 1990 Method of determination of leukocyte adherence inhibition. Authors Certificate No. 265601, Prague. [13] Jandová A, Nedbalová M and Trojan S 1997 LDH virus - parasite or energetic system of animals? Arch. Physiol. Biochem. 105 239
[14] Jandová A, Pokorný J, Kobílková J, Trojan S, Nedbalová M, Dohnalová A, Čoček A, Mašata J, Holaj R, Tvrzická E, Zvolský P, Dvořáková M and Cifra M 2009 Mitochondrial Dysfunction. Neural Netw. World 4 379-91