ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In-vitro Effects Of Modulated Ultrasound With Intensity Of 0.2 W/cm^2 On Pregnant Mares' Blood

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ABSTRACT

Characteristics of in vitro modulated ultrasonic effect in red blood cells (RBCs) from pregnant mares were studied. Blood samples from 3 to 5 ml in volume were sonicated with running ultrasonic wave of 0.88 MHz-frequency generation, modulation frequency of 10000 Hz, therapeutic intensity of 0.2 W/cm2 for 10-45 sec according to the author's technique [5]. The smears were made and stained with the standard Diff-quick method of differential staining of the biopreparations [6], followed by the analysis of the morphological state of the cells in the test and control (intact erythrocytes) by light microscopy. The authors of the article registered the ordered erythrocyte location, RBC micro- and macro-aggregation in the blood of pregnant animals, with simultaneous complete absence of signs of morphological changes in other blood cells. The results obtained demonstrated the possibility of directed ultrasonic impact on RBCs from pregnant mares and could be used as a basis for diagnostic methods of female pregnancy.

Keywords: ultrasound, modulation, mare, red blood cells.

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ISSN: 0975-8585

INTRODUCTION

One of the most widely used methods of veterinary diagnostics can be considered ultrasound. However, the analysis of international scientific databases of SCOPUS, Web of Science, e-Library and others showed a lack of literature on the use of low therapeutic insensitive ultrasound (US) in laboratory diagnosis of the state of animal cells and tissues. At the same time, many studies have been and are being conducted on ultrasonic effects in model objects and cell lines [1, 4]. Since the 90s, the features of acoustic control of individual cells and particles have been studied and the possibility of its application in practice is being tested [5]. Various mechanisms that lead to cell death or alteration have been investigated. The predominance of any of them is directly related to the change in US intensity. ***The purpose*** of investigation was to reveal the characteristic morphological features of erythrocytes (***RBCs***) from pregnant mares after a short-term exposure to a modulated ultrasound with the intensity of 0.2 W/cm^2 and the frequency generation of 0.88 MHz.

MATERIALS AND METHODS

To solve the problem, there were formed groups from the clinically healthy mares of different ages and breeds, according to the principle of analogues — background clinical, physiological, biochemical and hematological parameters. The work was in line with the "International Recommendations for Biomedical Research with Animals" and other veterinary and zoo-hygienic requirements. Ration fodders was according to state standard (P 5 025 892). During the research period, material from 18 horses was taken. For the experiments, the blood remaining from the clinical and biochemical planned animal surveys in the academy and its branches was used.

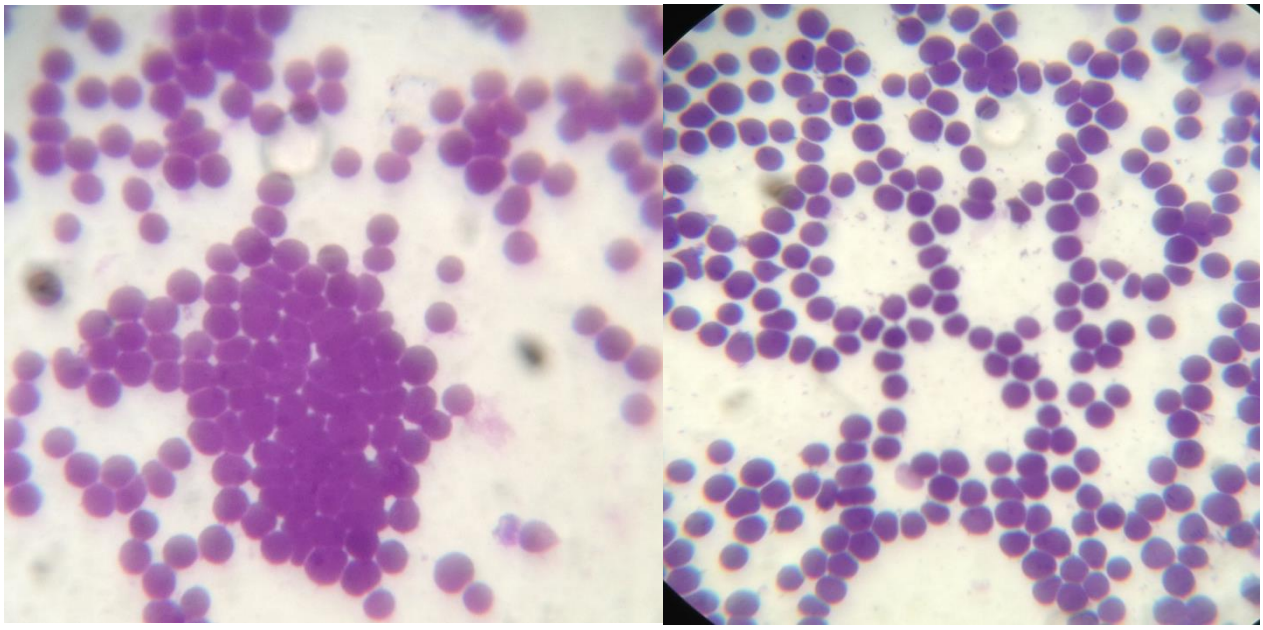
Blood samples volume from 3 to 5 ml was placed in a cuvette and sonicated for 10-45 sec (step of 5-15 sec) by modulated ultrasonic wave of 0.88 MHz, intensity of 0.2 W/cm^2 and frequency modulation of 10,000 Hz according to the author's technique [5]. Smears were made, stained with a standard method of differential staining of biological preparations Diff-quick [6], after which the analysis of the morphological state of cells was carried out using light microscopy ("LOMO", 10/0.25 lens, K10x18 ocular, immersion). When approbation of the method of targeted influence on ***RBCs***, 100 blood samples from mares with a gestation less than 1 month and 10 controls from non- pregnant mares were taken. At least 12 replicas were performed; after finding the effective mode from 3 to 10 smears per point were analyzed. The indicators before and after the exposure were compared. The statistical processing of the results was carried out using the application package "Statistica 6.0". The reliability of differences in mean values was determined using the t-test of Student; the differences were reliable at $P < 0.05$.

RESULTS AND DISCUSSION

Biological effects of ultrasound are multifactorial. The predominance of one of the mechanisms of biological action will depend on the type of cells, ultrasonic intensity, frequency and exposure time [1, 9, 10]. Under the acoustic waves effect on a living cell the effect of both change in the surface charge and in the functional state of the cells may be the result of the compression in the wave of cytoplasmic membranes and of the piezoelectric effect realization [4, 6]. These can reduce the number of active channels due to lateral diffusion of lipid bilayer molecules and can change the physiological state of the tissue [1, 4, 6].

Analysis of the results of sonication was based on the registration of physiology changes in the blood cells after US exposure (Figures 1a, b, 2) compared with the intact cells state (Figure 3). Microphotographs show the results of *in vitro* ultrasonic exposure of blood samples of pregnant (Figure 1) and non-pregnant (Figure 2) mares in comparison with control samples (Figure 3).

ISSN: 0975-8585



a *b*

Figure 1: Microphotographs of blood smears from pregnant mare (initial stage of pregnancy) after *in vitro* action by ultrasound with a 0.88-MHz generative frequency, therapeutic intensity of 0.2 W/cm^2, modulative frequency of 10,000 Hz. (Lens 10/0.25, ocular K10x18, Diff-quick stained) *a* — exposure 25 sec. Macro- and microaggregation of erythrocytes, "patterns" of *RBSs*, accumulation of cells. No destruction and anisocytosis; *b* — exposure 20 sec. *RBS's* microaggregation, formation of "patterns", chains and strings of erythrocytes.

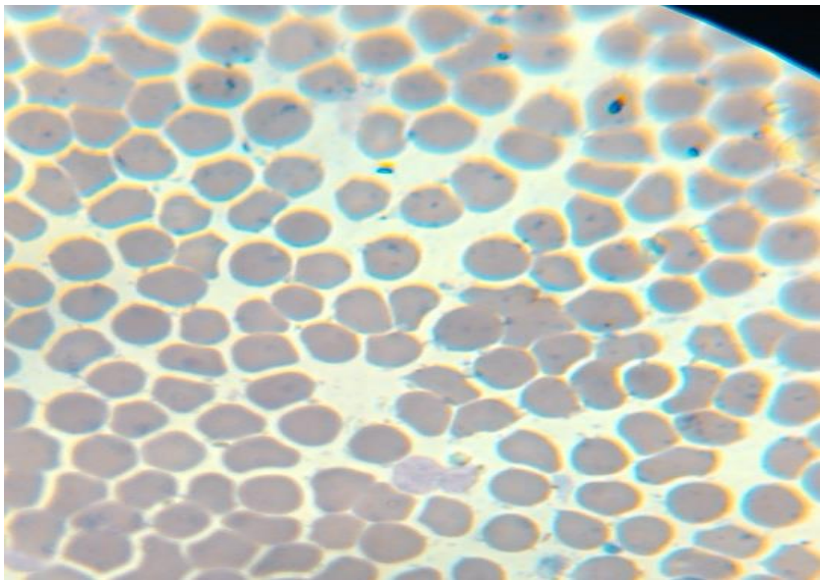


Figure 2: Photograph of a blood smear of non-pregnant mare. Parameters of *in vitro* 25-sec ultrasonic exposure of blood: 0.88-MHz generative frequency, intensity of 0.2 W/cm^2, modulative frequency of 10,000 Hz. (Lens 10/0.25, ocular K10x18, Diff-quick stained.) Blood cells without features.

ISSN: 0975-8585

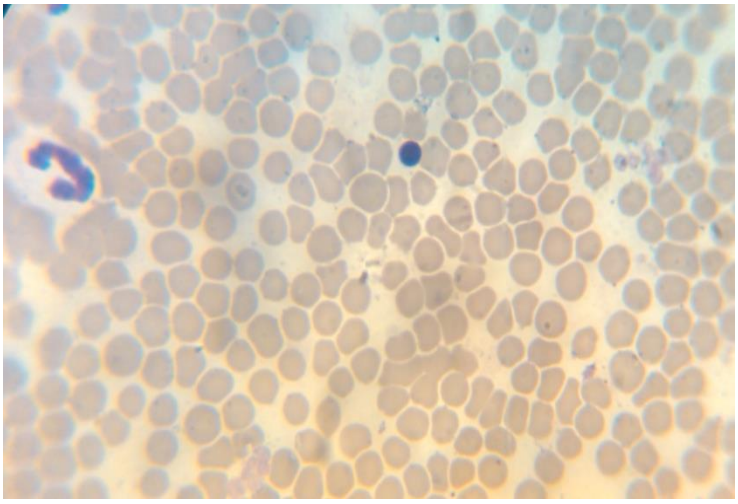


Figure 3: Photo of horse's blood smear. Control. Intact cells. Lens 10/0.25, ocular K10x18, Diff-quick stained.

Photo 1 (Figures 1a and 1b) shows the mare's blood cells at an early stage of gestation (2-–3 weeks) after treatment with a modulated ultrasonic wave at a frequency of 0.88 MHz, intensity of 0.2 W/cm^2, frequency modulation of 10,000 Hz, exposure time within 20–25 seconds. In the field of view of the microscope, micro- and macro-aggregation of erythrocytes, the formation of "patterns", cytochaines, and "lines" of erythrocytes are clearly visible. Cytolysis, destruction, aggregation and changes in the morphology of other blood elements were not detected. ***RBCs*** had a normal shape, the area was unchanged, aniso- and poikilocytosis were not recorded. US-exposure by the same parameters of non-pregnant mare's blood did not result in erythrocyte aggregation (Figure 2). Before us M.W. Miller and colleagues noted the reaction of human lymphocytes and erythrocytes taken at a concentration of 2.5 x 10^6 cells/ml on 60-second sonication [6]. It was shown that after the action of continuous ultrasound, 10%-increase in lymphocytolyses over RBS-lyses was obtained. That fact was explained by the size of the blood cells. However, in our experiment with samples of the native blood of pregnant and non-pregnant mares, only ***RBCs*** reacted to the sonication in a definite manner. Probably, the features revealed are associated with the onset of the viscosity changes in the blood and of course with general change in the biochemical composition of the blood of pregnant animals in the initial stages of pregnancy. Later, to confirm the pregnancy of mares from the experimental group data from a later rectal examination and data from artificial insemination logs were used.

One of the most urgent tasks of veterinary medicine is to increase efficiency and to reduce labor intensity of early pregnancy diagnosis of productive animal's females. Veterinarians use widely the rectal method of pregnancy diagnosis. But it is impossible to determine the early terms of pregnancy rectally. Laboratory methods of early recognition of mare's pregnancy include urine folliculin-content analysis & progesterone analysis. The latter can identify with certainty only infertility, when the concentration of this hormone remains below 1 ng/ml [2, 3]. The above-mentioned laboratory methods are rather difficult, complex, highly costly and require the use of special equipment, laboratory animals and test kits. However, the techniques can't be called highly specific. The features of the physiology of blood cells of mares in the ultrasonic field will allow to develop a laboratory initial-stage's pregnancy diagnostical method based on the ***RBC's*** functional state changing caused by the action of amplitude-modulated ultrasound of therapeutic intensity on blood cells *in vitro*.

CONCLUSION

The optimal conditions for irradiation of animal blood samples were found: exposure 20–25 sec, intensity of 0.2 W/cm^2 and frequency modulation 10 000 Hz, leading to a change in the state (selective aggregation) of ***RBCs*** of pregnant mares while in unfertile mare's cell morphology and in the distribution of ***RBCs*** in the field of view of the microscope remains without specific features, within the limits of the normative indices. Moreover, the erythrocyte aggregation occurs without the appearance of signs of cytolysis, against the background of the absence of aniso- and poikilocytosis and with simultaneous complete absence of changes in the morphology of other blood cells. The features of the physiology changes in the blood cells of

pregnant mares will allow to develop a method for laboratory diagnostics of initial stages of pregnancy by changing the cytomorphology caused by *in vitro* action of amplitude modulated ultrasound of low therapeutic intensity in blood cells.

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