

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Physiology of Blood Cells of Pregnant Mares in Modulated Ultrasonic Field.

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ABSTRACT

Characteristics of the modulated ultrasound effect (modulation frequency 1100 Hz, intensity 0.05 W/cm²) on white blood cells (WBCs) from pregnant mares were studied. The authors of the article registered *in vitro* lymphocyte aggregation, appearance of signs of nuclear deformation / lysis as well as plasmolyze in the blood of pregnant animals, with simultaneous complete absence of signs of cellular destruction in other form elements. The results obtained demonstrated the possibility of directed US-impact on different WBC forms from foal mares and could be used as a basis for diagnostic methods of female pregnancy.

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

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INTRODUCTION

The urgent task of veterinary medicine is to increase the efficiency and reduce the labor intensity of early pregnancy diagnosis of productive animals' females. The early diagnosis of pregnancy is important in the struggle to increase the percentage of stinginess, allowing, according to the diagnosis results, either to stop the mare's mating pregnant, but showing false hunting, or continue the mating, applying measures to prevent the abandonment of the mare unfertilized. Studies conducted from the 1960s to the 2010s showed that amplitude-modulated ultrasound (US) used on biological tissue is more efficient than continuous wave [1-5].

The purpose of this study was to detect possible changes in the functional state of WBCs of foal mares *in vitro* exposed to modulated ultrasound of minimal therapeutic intensity.

MATERIALS AND METHODS

In our study, no animal was harmed. Purposes, methodology and principles of our operations didn't include *in vivo* experiments on living beings. The experimental *in vitro* work was carried out at the Department of Information Technology, Mathematics and Physics of Moscow State Academy of Veterinary Medicine and Biotechnology. All animals were adult and healthy. **Groups of animals:** 9 horses aged from 2 to 8 years with a pregnancy up to 1 month and 5 "unwed" mares. Animals' blood was exposed to US with a space-averaged, time-averaged (SATA) intensity of 0.05 W/cm^2 during a period from 15 to 45 seconds, with modulation frequency of 1100 Hz and the carrier frequency of 0.88 MHz, according to the previously proven technique [6]. US therapy medical devices were: UST-1-01 F and UST-1.02 S, combined with thermostat U7^c. We applied modulator GZ-112. We consider it inhumane to receive regularly a large amount of blood from animals for the extended biophysical series of experiments for reliable statistics. Therefore, the sonication technique has been specifically designed and tested in samples with the minimum volume. We adjusted the US exposure for each blood volume to receive comparable results. Blood samples of 3-5 ml were sonicated under identical conditions (oscillator square, cooling fluid, circulative rate). A coolant, distilled water, circulated continuously (so-called "flow-through cooling").

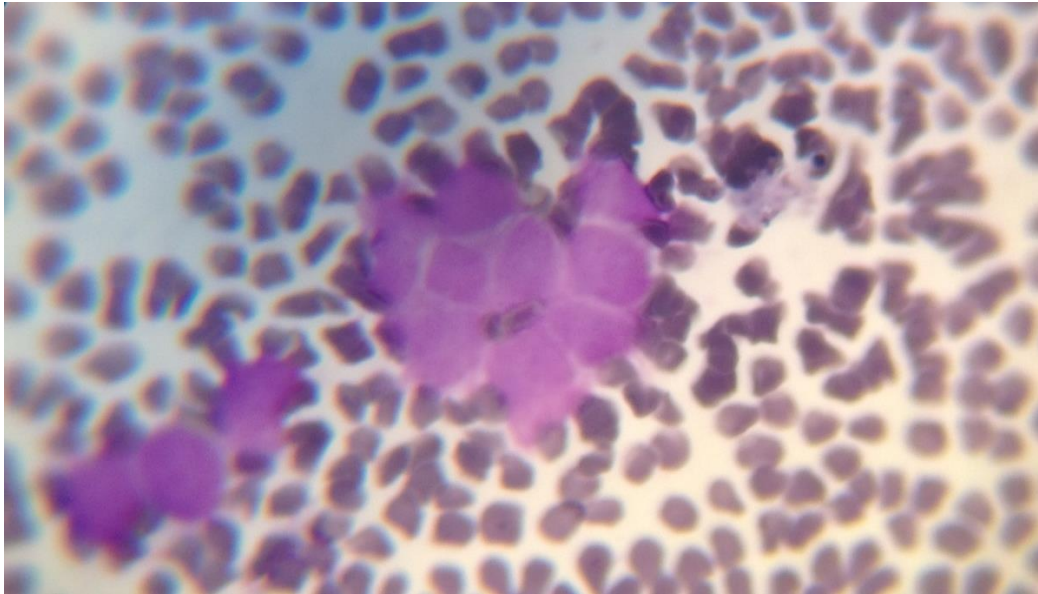
Blood smears and Cytochemical methods

US effects on WBCs were observed under a light microscope. Blood of the same animal, untreated (intact specimens) served as a control on determining the US effect on WBCs. Blood smears (control and after the US exposure) were examined under immersion in a transmitted light microscope «Mikmed-5» (optical objective – $100\times/1.25$; ocular lens – $10\times/18$).

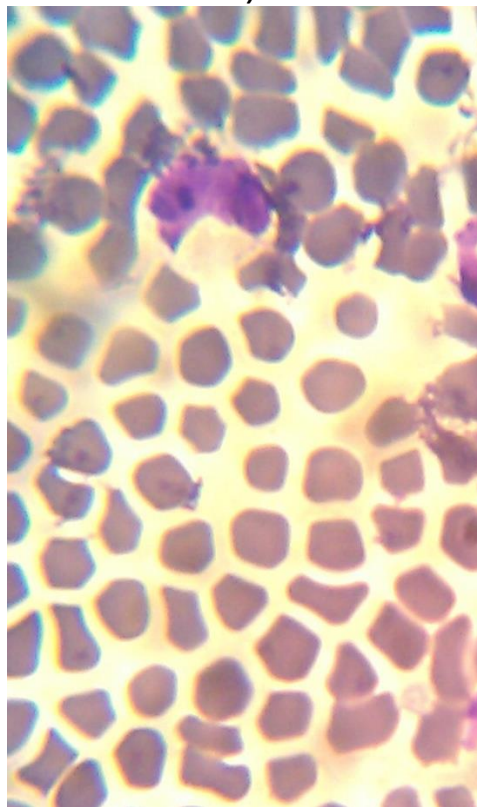
Smears were made and stained according to the DIFF-QUICK technique [7]. Each sample was examined at least 10 times. Statistical results processing was performed with the use of "Statistica 6.0" program. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The *in vitro* analysis of the physiological state change of the blood cells was carried out on the basis of recording changes in cell morphology after US exposure (Figures 1-2) and the physiological state of intact blood cells (control, Fig. 3). Photomicrographs 1-3 show the results of a 30-second sonication of blood from pregnant (Figure 1 (a, b)) and unfertilized (Figure 2) mares versus control samples (Figure 3). Photo 1 shows the picture of lymphocyte aggregation after irradiation of blood samples of pregnant mares in the initial stages: ultrasound intensity of 0.05 W/cm^2 , a modulation frequency of 1100 Hz, exposure 30 sec). The gestation period was 2-3 weeks. The aggregation of all types of lymphocytes (large, medium (Figure 1a) and small (Fig. 1b)) was recorded. When ultrasound exposure to the same parameters on the blood of ungainly mares, cellular aggregation was absent (Figure 2).



a)



b)

Figure 1: (a, b). Photo of the mare's blood smear in the initial stages of the pregnancy. Aggregation of lymphocytes after irradiation of samples with ultrasound of intensity 0.05 W/cm^2 , modulation frequency 1100 Hz, for 30 secs: a) – aggregation of large and medium lymphocytes; b) – aggregation of small lymphocytes. (Photo by A. A. Oleshkevich)

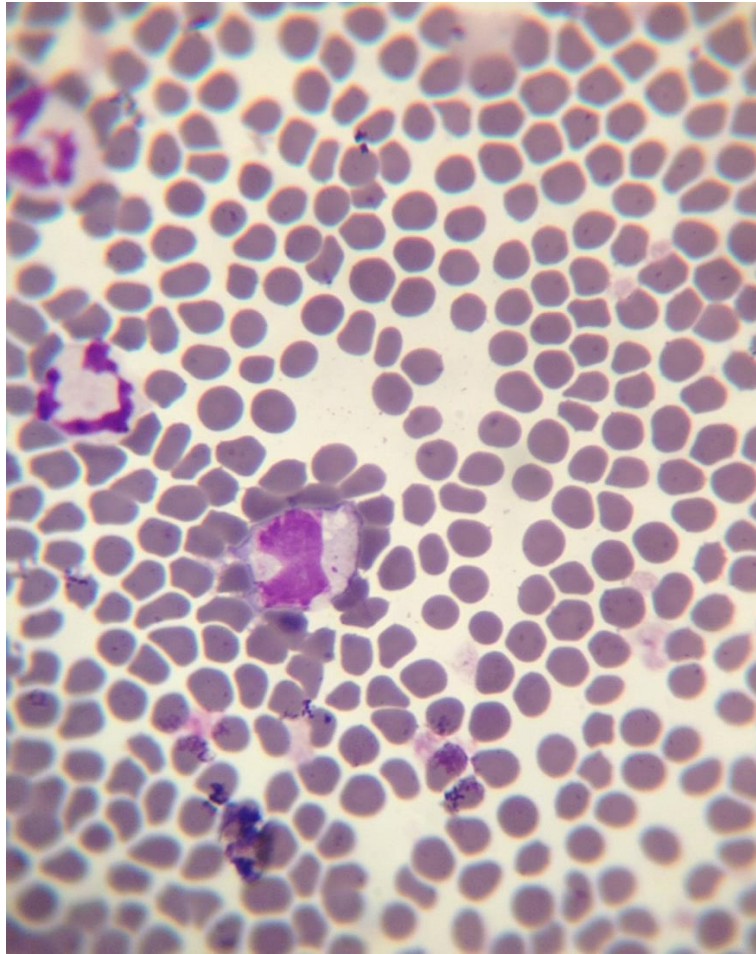


Figure 2: Microphotograph of a blood smear is unmarried ("idle") mare. The sample after ultrasound irradiation: intensity of 0.05 W/cm^2 , modulation frequency of 1100 Hz, for 30 seconds (Photo by A. A. Oleshkevich)

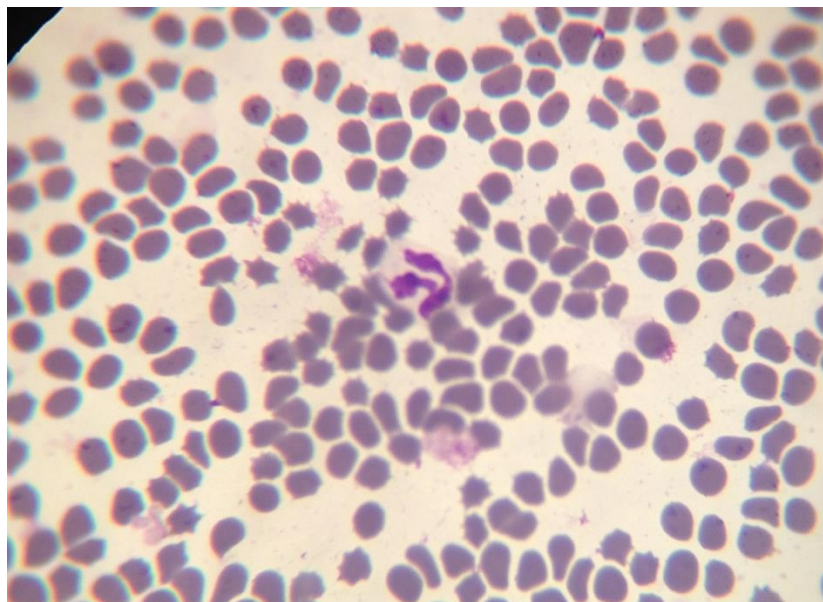


Figure 3: Photo of mare's blood smear. Control, intact cells (Photo by A. A. Oleshkevich)

To confirm the pregnancy the data from a later rectal examination and data from the magazines of artificial insemination were used.

Thus, the studies conducted reliably show the deterioration of hematological indices and the presence of regular changes in the physiology of mare's blood cells caused by the action of ultrasound of certain intensity and frequencies on blood samples. The most valuable diagnostic signs are the increased aggregation of WBC, i.m. lymphocytes, which can be used for early diagnosis of pregnancy in animals. Probably, the revealed features are associated with a change in the viscosity of blood of pregnant animals in the initial stages and, of course, a general change in the biochemical composition of the blood.

Practical significance of the results

- the regularities of the interaction of blood cells of pregnant and nonpregnant animals with ultrasound of a safe range — the minimum of the applied therapeutic intensity and low exposure time - were revealed;
- the possibility of creating of a new method for initial stages mares' pregnancy detection is established by determining of the cytological changes in WBC and the features of the cellular response to the action of acoustic (US) waves of low therapeutic intensity *in vitro*;
- the arsenal of ways to diagnose pregnancy in horses is expanded;
- reliability of diagnosis of the initial stage of pregnancy, when the clinical picture is still not clearly expressed, increased.

REFERENCES

- [1] Andrianov V. The biological effect of ultrasound waves and ultra-high frequency electromagnetic oscillations. Morphological changes in the testes caused by ultrasound exposure. Kiev: "Naukova dumka"; 1964. [Book in Russian].
- [2] Hwang J.H., Tu J., Brayman A.A., Matula T.J., Crum L.A. Correlation between inertial cavitations dose and endothelial cell damage in vivo. *Ultrasound Med Biol* 2006; 32(10): 1611-9.
- [3] O'Brien W.D. Jr, Brady J.K., Dunn F. Morphological changes to mouse testicular tissue from in vivo ultrasonic irradiation (preliminary report). *Ultrasound Med Biol* 1979; 5(1): 35-43.
- [4] Sarvazyan A.P., Belousov L.V., Petropavlovskaya M.N., Ostroumova T.V.. The action of low-intensity pulsed ultrasound on amphibian embryonic tissues. *Ultrasound. Med. Biol*, 1982; 8(6): 639-54.
- [5] Various *in vitro* Effects of Continuous and Modulated Ultrasound on Blood Cells of Different Animal Species. *Biophysics*, 2017. 62(4). 603-615.
- [6] Oleshkevich A., Kuzmina T., Komarova E. Blood cells state analysis after exposure to acoustic (ultrasound) waves. *Proceedings of the Conf. "Theoretical and Applied Problems of Education and Science"*. Tambov 2014; 12:137-138. [Article in Russian].
- [7] Oleshkevich A., Studies of frequency-dependent changes under modulated ultrasound exposure on cells in suspension. *International Journal of BioMedicine*. 2015. 4(1). 30-34.