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# HEMATOLOGICAL AND BIOCHEMICAL BLOOD INDICATORS OF YOUNG GILTS AFTER ESTRUS SYNCHRONIZATION

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***Abstract***

*Synchronization and stimulation are used to obtain large numbers of embryos. Therefore, the studies carried out made it possible to assess homeostasis in the dynamics of metabolism in different phases of regulation of the reproductive cycle when using the proposed drug "Estrosynchron". The drug blocks the secretion of pituitary gonadotropins, which inhibits the growth of follicles and the ovulation process and, accordingly, the manifestation of the phenomena of the sexual cycle. It was found that over the period (18-20 days) of feeding Estrosynchron in gilts after treatment, blood parameters decreased: total protein, globulin fraction, α1-globulin, α2-globulin fraction compared with the indicators before treatment. After treatment, there was an increase in the number of erythrocytes, hematocrit, platelet count and thrombocyte, as well as a significant increase in the average hemoglobin content in an erythrocyte and a decrease in the rate of erythrocyte coagulation. At the same time, studies of blood parameters provide additional information about the characteristics of breeding pigs, depending on the age and physiological state during reproductive use.*

***Key words**: estrosynchron, estrus synchronization, gonadotropins, homeostasis, leukogram, stimulation.*

## INTRODUCTION

Modern pig breeding requires intensive reproduction, stimulation and synchronization of the sexual function of gilts to replenish the main herd and planned predictable artificial insemination. In breeding farms, successful stimulation and estrus synchronization are produced when gilts reach a weight of 120-130 kg and have 2-3 sexual cycles before the start of treatment (Melnik et al., 2017; Melnik, 2018).

Fundamental and applied research in the field of physiology, nutrition, genetics, animal behavior, environment and management over the past 20 years has laid the foundation for the development of highly productive females and various management methods and technologies that have significantly increased the efficiency of reproduction in the breeding herd (Christiansen, 2005; Kraeling, & Webel, 2015; Kramarenko, et al., 2020).

Optimum reproductive performance are achieved through a variety of strategies to meet the high productivity expectations of modern farms. Hormones have been used to control the reproductive function of sows and gilts in various protocols in order to optimize management practices and increase the overall efficiency of pork production (Ziecik et al., 2021).

Also, synchronization and superovulation are used to obtain a large number of embryos for experimental and practical purposes, but with mixed results. In their studies Ziecik et al. (2005) compared the quantity, quality and in vitro development of embryos obtained from pigs after synchronization of single or double estrus and superovulation. The single and double hormonal stimulation regimens resulted in an increased number of embryos with a higher percentage of embryos classified as degenerated compared to the control group without stimulation. The number of embryos to be cultured did not differ between single and double synchronized groups. The highest percentage of embryos obtained was observed in the control group, where no stimulation was performed. Scientists have confirmed that synchronization and superovulation is sufficient to obtain a large number of embryos, however, both methods of synchronization resulted in a significant number of degenerated embryos (Ziecik et al., 2005).

Pharmaceuticals such as progesterone analogs and gonadotropins are commonly used in practice in a number of countries to improve the reproductive function of gilts and sows. Data from studies by Ziecik et al. (2021) show that gonadotropins used to control the reproductive

function of sows and gilts, which depends on sexual maturity, affect endocrine and molecular environment of preovulatory follicles.
Various exogenous hormones can be used to control follicular development and synchronize ovulation in sows and gilts. Estrus can be induced by ECG administration with or without concomitant HCG, the latter being analogous to LH. The use of HCG is likely to be relatively more effective in conditions of limited endogenous LH, for example, in young females, because in pigs it is LH that is the main driver of follicular development from the middle follicle stage to the follicle stage ovulation point (Abbara, et al., 2018; Caárdenas & Pope, 2002; De Rensis & Kirkwood, 2016).
De Rensis & Kirkwood (2016) dealt with the problems of estrus control and estrus synchronization in gilts and sows. Studies have shown that in females with a regular cycle, estrus can be controlled and synchronized by blocking follicular growth at the mid-follicle stage by suppressing endogenous LH achieved by feeding the progestogen altrenogest.
This effectively stops the follicular phase and when altrenogest is withdrawn, the follicular phase begins.
The use of gonadotropins to synchronize the reproductive cycle in pigs was used in the studies by Day et al. (1965). The fertility rate of the first estrus after gonadotropin treatment was higher than the fertility rate of comparable untreated animals in terms of fertilization rates, the mean number of embryos at 25 days of gestation, and litter size at farrowing.
The procedure for using gonadotropin injections to synchronize reproductive cycles in pigs provides an effective and reliable means of reducing reproductive timing variability without compromising subsequent reproductive capacity (Day et al., 1965).
Quirino et al. (2020) also confirm that the estrous cycle can be controlled by hormones such as progestogens to suppress the follicular phase, allowing follicular growth to resume after cancellation hormones.
Altrenogest is currently the only commercial progestogen available in pigs and its efficacy in controlling the estrous cycle in pigs is well documented, resulting in over 79% estrous expression within 3-8 days after hormone withdrawal.

## MATERIALS AND METHODS

Experimental studies were carried out in the conditions of pedigree farms in Nikolaev, Kherson, Odessa regions of Ukraine.
To fulfill the assigned tasks, scientific and scientific-economic experiments were carried out, in which 1468 pigs were used, including 16 young pigs.
For the experiment, according to the principle of analogues, groups of sows of different breeds and combinations bred in Ukraine were formed: the large white pig breed (LW), which is universal and the most numerous, was taken for control. The following breeds were used for research: Landrace (L), Duroc (D), Red White-belted (RWB), Pietrain (P), Ukrainian Meat (UM) and F1 local pigs (LW × L) and other combinations.
Hematological studies were carried out on a complex of blood morphological composition, biochemical and hormonal parameters (Kondrakhin et al, 1985; Vlizlo, et al, 2012).
Pig blood samples were taken from the jugular vein using five- or ten-gram special disposable syringes of the "Monovet" type, which is a closed system consisting of a syringe-test tube and an injection needle. In the white "Monovet" there are plastic balls with a blood coagulation activator, due to which the blood quickly coagulates and a clear border is formed between the clot and the serum, in the red one there is heparin, which prevents blood clotting. Monovet is used for transportation, as a container and as a test tube for centrifugation. The animals were fixed in a standing position behind the upper jaw with a loop of a flexible steel cable fixed in a metal casing (Levchenko et al., 2004). The site for puncture of the jugular vein was determined in the groove formed by the long muscles of the neck, to the right or to the left, five centimeters cranial from the sternum. The Monovet needle was inserted from the bottom up and with an inclination in the caudomedial direction to the appropriate depth depending on the age, weight and breed of animals. The penetration of the needle into the lumen of the vein allows the syringe to be quickly filled with blood (Rybalko et al., 2005). Blood tests were performed on an automatic hematology analyzer BC-3000 pluse (Mindrey) for counting and measuring the sizes of WBC,

RBC, PLT and measuring HGB by the electrical impedance method, based on measuring changes in electrical resistance generated by a particle passing through the aperture. Since blood cells are not light conductive, the moment they pass through the aperture, they cause an increase in impedance, which is in direct proportion to the size of the cells (Levchenko et al., 2004).

The determination of hormones and enzymes in pig blood serum was carried out on an automatic analyzer ChemWell (2in1) Awareness technology 1nc (USA), which is used to determine 200 biochemical and 170 enzyme-linked immunesorbent tests, provides simultaneous use of 40 reagents with the support of Windows software (Kondrakhin et al., 1985; Vlizlo et al., 2012).

An automatic universal analyzer Vitalab was used to determine total protein using a Liguick Cor-Total PROTEIN diagnostic kit for determining cholesterol using reagents Bio Systems S.A. (Spain), for measuring the concentration of CPK using the reagent Creatine Kinase (CK) Bio Systems S.A. (Spine) (Rybalko, et al, 2005).

Protein and lipid electrophoresis was performed using a UEF-01 "Astra" instrument system. A set of reagents was used to determine the protein fractions of blood serum by electrophoresis on cellulose acetate membranes, followed by densitometric determination of protein fractions (Christiansen, 2005).

## RESULTS AND DISCUSSIONS

In sexually mature gilts, the lability of homeostasis is aimed at maintaining the rhythmic stages of the estrous cycle to create optimal conditions for the fertilization of eggs. Monitoring data on the homeostasis of the body of pigs after synchronization of sexual estrus showed insufficient research in the field of the relationship between blood parameters and the administration of gonadotropins. Therefore, the studies carried out made it possible to assess homeostasis in the dynamics of metabolism in different phases of regulation of the reproductive cycle when using the proposed drug "Estrosynchron". The drug blocks the secretion of pituitary gonadotropins, which inhibits the growth of follicles and the ovulation process and, accordingly, the manifestation of the phenomena of the sexual cycle (Melnik et al., 2017; Melnik, 2018).

The results of the study of hematological and biochemical parameters of the blood of pigs subject to stimulation and synchronization of sexual estrus are shown in Tables 1-3. The data obtained indicate that for the period (18-20 days) of feeding the drug in gilts after treatment, blood parameters decreased compared to the period before treatment: total protein by 3.7 g / l, globulin fraction by 1.9%, α1- globulin - 0.5%, which is significant ($p < 0.05$) compared to the indicators before treatment, and the α2-globulin fraction – by 1.7% ($p < 0.001$). A slight decrease in the observed β-globulin fraction – 0.2%, as well as cholesterol – by 0.13 mmol / L. Analysis of the data showed an increase in the albumin fraction by 1.9% at $p < 0.05$ and γ-globulin - 1.0%, respectively, the A / G ratio increased by 0.06, and the amount of β-lipoproteins increased by 1.2 at. units (Table 1).

This difference can be explained by a slight change in homeostasis under the influence of the drug. As a result of observing the behavior of the pigs while feeding the drug, its calming effect was established, the pigs rested more, during this period the increase in live weight increased and they did not show signs of estrus.

Earlier studies Burton & Westphal (1972) also revealed a relationship between blood biochemical parameters, before and after estrus synchronization, in rats and their behavior. Scientists have identified proteins that form dissociative complexes with circulating steroid hormones in the serum: albumin, the most abundant plasma protein, and highly specific glycoproteins found in low concentrations: corticosteroid-binding globulin (CBG or transcortin), the sex steroid binding protein (SB) and progesterone-binding globulin (PBG)

Table 1. Blood biochemical parameters of gilts, subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S\overline{x}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Total protein, g/l | 65.1±0.81 | 61.4±1.07* | -3.7 |
| Albumin, % | 40.2±0.59 | 42.1±0.71* | 1.9 |
| Globulins, % | 59.8±0.59 | 57.9±0.71* | -1.9 |
| $\alpha_1$-globulins, % | 5.3±0.15 | 4.8±0.14* | -0.5 |
| $\alpha_2$-globulins, % | 15.2±0.29 | 13.5±0.26*** | -1.7 |
| β-globulins, % | 17.5±0.30 | 17.3±0.54 | -0.2 |
| γ-globulins, % | 21.7±0.68 | 22.7±0.78 | 1.0 |
| Coefficient A / G | 0.68±0.031 | 0.74±0.033 | 0.06 |
| Cholesterol, mmol / l | 2.59±0.07 | 2.46±0.09 | -0.13 |
| β-lipoproteins, u.u. | 17.9±0.71 | 19.1±0.73 | 1.2 |

*Marks:* $^{*}$ P≤0,05, $^{**}$ P<0,01, $^{***}$ P<0,001

After treatment, starting from day 16, the rats were more mobile and active. Albumin interacts primarily through hydrophobic bonds; the constants of steroid complexes with specific globulins are several orders of magnitude higher. Estrogenic hormones increase CBG and SBP levels; androgenic hormones have the opposite effect. In rats, the inhibitory effect of corticosteroid hormones on CBG was observed; adrenalectomized rats have increased CBG activity (Burton & Westphal, 1972).

The hematological blood parameters of gilts subject to estrosynchron synchronization (Table 2) also had some difference, but in most cases it was not reliable.

Table 2. Hematological parameters of the blood of gilts subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S\overline{x}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Erythrocytes, $10^{12}$/l | 6.3±0.11 | 6.6±0.15 | 0.3 |
| Hemoglobin, g/% | 12.2±0.18 | 12.1±0.26 | -0.1 |
| Hematocrit, % | 35.5±0.84 | 37.4±0.93 | 1.9 |
| Average erythrocyte volume, fl | 58.0±0.63 | 56.4±0.72 | -1.6 |
| Average hemoglobin content in erythrocyte, pg | 15.9±0.14 | 17.8±0.11*** | 1.9 |
| Average concentration of hemoglobin in erythrocytes, g/l | 329±3.01 | 325±2.39 | -4 |
| The width of distribution of erythrocytes by volume, % | 17.8±0.27 | 17.6±0.22 | -0.2 |
| Erythrocyte sedimentation rate, mm / year. | 3.8±0.42 | 1.6±0.31*** | -2.2 |
| Platelets, $10^9$/l | 251±19.5 | 267±27.7 | 16 |
| Average platelet volume, fl | 9.7±0.12 | 9.2±0.15 | -0.5 |
| Platelet distribution width by volume, % | 15.1±0.11 | 15.0±0.12 | -0.1 |
| Thrombokrit, % | 0.238±0.029 | 0.247±0.031 | 0.009 |

*Marks:* $^{*}$ P≤0,05, $^{**}$ P<0,01, $^{***}$ P<0,001

An increase after the treatment of erythrocytes by 0.3 × 1012 / l, hematocrit by 1.9%, the number of platelets – 16.0 × 109 / l and thrombocrit by 0.09% was found. We have established a significant increase (p <0.001) in the average hemoglobin content in an erythrocyte by 1.9 pg. For other indicators, there was a slight decrease, but it is necessary to note a significant (p <0.001) decrease in the rate of erythrocyte coagulation by 2.2 mm / h. With a normal platelet count, this phenomenon can be explained by medication, i.e. using Estrosynchron.

Also in the studies of Engovatov et al. (2011) it was proved that stimulation of estrus in gilts increases the content of erythrocytes and hemoglobin in the blood of piglets, which leads to a higher preservation of the litter and their increased viability. At the same time, there was no difference in the content of leukocytes in the blood, but there was a certain tendency to an increase in the amount of total protein in the blood serum, which occurred mainly due to globulin fractions, namely, due to the content of the fraction of γ-globulins characterizing the protective functions of the body. Consequently, the use of biostimulants for sexual heat in gilts leads to more intense redox processes in their body.

As a result of our studies, a comparison of the effect of the drug on the natural resistance of gilts before treatment and after feeding it was calculated a blood leukogram, presented in Table 3. Blood leukocytes are responsible for protective processes in the body of pigs, the production of antibodies, phagocytosis, and neutralization of toxins

Table 3. Leukogram of the blood of gilts subject to estrus synchronization by "Estrosynchron", n = 16, $\overline{X} \pm S_{\overline{x}}$

| Index | Before treatment | After treatment | Difference (±) |
|---|---|---|---|
| Leukocytes, $10^9$ | 14.9±0.68 | 15.0±0.72 | 0.1 |
| Eosinophils, % | 3.3±0.61 | 3.3±0.52 | - |
| Neutrophils, % | | | |
| - stab | 5.1±0.49 | 3.7±0.40* | -1.4 |
| - segmented | 37.4±1.96 | 36.7±2.38 | -0.7 |
| - juvenile | - | - | - |
| Lymphocytes, % | 50.8±2.34 | 53.5±2.68 | 2.7 |
| Monocytes, % | 3.6±0.27 | 2.7±0.38 | -0.9 |
| Basophils, % | - | - | - |
| Lymphocyte/neutrophil ratio | 1.20 | 1.32 | 0.12 |

*Marks:* * P≤0,05, ** P<0,01, *** P<0,001

The data in Table 3 indicate that a significant difference in blood leukogram indicators of gilts before and after treatment has not been established, except for a decrease in the number of stab neutrophils by 1.4% (p <0.05).

An increase in the number of lymphocytes by 2.7% was found, but the difference was not significant. Using the leukocyte ratio of lymphocytes and neutrophils as a test, an increase of 0.12 was found, indicating a slight stress response in the gilts.

Similar data were obtained by Romanenko, (2015), for example, the marked insignificant lymphocytosis (by 3.6%), possibly indicates a slight decrease in the microphagocytic function of the body.

And an increase in the number of segmented neutrophils by 27.3% helps to increase the body's defenses to intoxication.

Kmieć & Terman (2006), Kondruchina et al. (2021) in their studies also confirm that the adverse effect on the animal's body is reflected in the hematological profile, and its indicators indicate the state of nonspecific resistance of the organism, which leads to reduced reproductive function. And they recommend the synchronization of sexual activity with biological products, as one of the ways to prevent the negative impact of stress factors on the body.

## CONCLUSIONS

Thus, it was found that for the period (18-20 days) of feeding Estrosynchron in gilts after treatment, blood parameters decreased: total protein by 3.7 g / l, globulin fraction by 1.9%, α1-globulin - 0.5 %, which is significant (p <0.05), α2-globulin fraction - by 1.7% (p

<0.001) in comparison with the indices for treatment. An increase after the treatment of erythrocytes by 0.3 × 1012/l, hematocrit by 1.9%, the number of platelets - 16.0 × 109/l and thrombocyte by 0.09%, as well as a significant (p <0.001) increase in the average the hemoglobin content in the erythrocyte by 1.9 pg and a decrease in the rate of erythrocyte coagulation by 2.2 mm/h (p <0.001). And studies of blood parameters provide additional information about the characteristics of breeding pigs, depending on age and physiological state during reproductive use.

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