**Dear colleagues!**

The modern trends of biological threats growing, emergent diseases (Lumpy skin disease, Foot-and-mouth disease, African swine fever, Avian influenza and other in Europe and Asia) determine the necessarily to pay the extremely high attention to the biosafety issues and biological hazards control.

The National Scientific Center 'Institute of the Experimental and Clinical Veterinary Medicine' is the leading specialized research institution in Ukraine created for solving scientific and practical tasks of veterinary animal. NSC IECVM's basic research are focused on: immunogenesis and disease pathogenesis, indications, authentications, isolations and studies of biological features of their causative agents, developments of facilities and systems of monitoring, diagnostics, prophylaxis and prognostication of infectious diseases of animals, monitoring of quality and unconcern of agricultural produce and development of the normative basis for animal diseases control and biosafety. NSC IECVM coordinates implementation of scientific researches on questions veterinary medicine, that conduct scientific establishments of NAAS, State Service of Ukraine for Food Safety and Consumer Protection, and Higher educational establishments of Ukraine of agrarian profile.

New journal 'Journal for Veterinary Medicine, Biotechnology and Biosafety", discovered in 2015, aimed to consolidate and share the new developments and achievements in the area of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. Our journal promotes the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community. It includes cooperative veterinary and medical aspects, fitting to One Health Approach declared by WHO, OIE, and FAO. It was included in Index Copernicus and eLibrary scientific databases.

The Editorial board hopes, that our issue will be interesting for wide auditorium of scientists and practical specialists in veterinary medicine, biology, biotechnology and biosafety. We invite new authors for fruitful collaboration and joint development.

**Sincerely yours,**
**Editors-in-Chief**

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Prof. Anton GERILOVYCH

# GUIDELINES FOR THE PREPARATION OF THE PAPERS SUBMITTED FOR PUBLICATION AT THE 'JOURNAL FOR VETERINARY MEDICINE, BIOTECHNOLOGY AND BIOSAFETY'

1. Papers must be submitted in an electronic variant and should be sent directly to the editorial board at nsc.iecvm.kharkov@gmail.com or inform@vet.kharkov.ua with subject 'Article in JVMBBS'

2. Papers must be written in English

3. Authors make sure there are no typographical errors in the manuscript

4. Papers must be presented in Word format, in an A4 layout, using Times New Roman 14 point font, which should be single-spaced with 25 mm margins

5. Tables and illustrations must be must be submitted as separate files and inserted in the text

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(e) Summary in English (between 200 to 300 words), which should be included: the aim of the work, materials and methods, the results of the work, conclusions

(f) Keywords (up to 8)

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**NATIONAL SCIENTIFIC CENTER 'INSTITUTE OF EXPERIMENTAL AND CLINICAL VETERINARY MEDICINE'**

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## DIAGNOSTICS OF METABOLIC DISORDERS IN THE COWS' ORGANISM BY BASIC BIOCHEMICAL BLOOD MARKERS: EVIDENCE FROM FP 'MRIIA' (RIVNE DISTRICT, RIVNE REGION, UKRAINE)

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**Summary.** The purpose of the research was to determine the characteristics of metabolic disorders in dairy cows in the dry periods, calving, and after calving periods in FG 'Mriia' in Rivne district of Rivne region. Researches were conducted in FG 'Mriia' v. Velyka Omeliana of Rivne district, Rivne region on cows of Ukrainian black-rumped dairy breed. The presence of metabolic changes in the cows in dry period (n = 6), post-partum cows (n = 8), and cows 10 days after calving (n = 8) were determined by biochemical parameters (markers) in blood serum samples, which were tested by conventional biochemical methods using the 'Cobas c 311' analyzer, and the content of inorganic elements was determined using the 'C-115M1' atomic absorption spectrophotometer. According to the results of obstetric examination of sick animals, carried out in the dry season, such diseases as ketosis (8.9%), fatty liver syndrome (6.7%), and udder edema (5.6%) were determined. In cows, the level of ketone bodies was 2.0 ± 0.04 mmol/l. 75.0% of animals diagnosed with ketosis in the dry period were prone to develop other diseases such as ovarian cysts and postpartum endometritis. Idiopathic diseases averaged 12.2%. Among the concomitant pathologies, the detention of litter was detected — 32.2%. 25 cases (27.8%) of post-partum paresis were recorded out of 90 cows examined. The following metabolic disorders have been established by biochemical markers in animals of the respective groups, namely: in cows in the dry period — a decrease of the total protein level along with its globulin fraction, the decrease of glucose content relative to the reference values of these indicators; in cows in the calving group on the background of changes in the proteinogram — a decrease in total cholesterol was determined; and in cows 10 days after calving — a decrease in the content of albumin relative to the reference values of these indicators was determined. As a result of determining the content of inorganic elements, it has been established: in the cows in dry period group — lack of zinc, copper, manganese, selenium, iodine, and cobalt and excess of iron and nickel; in the group of calving cows — there is a lack of zinc, copper, selenium, and iodine and excess of iron; in the group of cows 10 days after calving — lack of zinc, manganese, selenium, and cobalt. The data obtained can be interpreted to diagnose metabolic disorders in other farms and to perform corrective measures

**Keywords:** metabolic disorders, diagnostics, cows, serum, biochemical markers, inorganic elements

**Introduction.** Metabolic diseases in cattle in modern conditions of animal husbandry occupy one of the dominant places in the structure of non-contagious pathology (Alekhin, 2011; Kovalenko et al., 2015; Urazaev et al., 1990). The detection of metabolic disorders, especially in highly efficient cows, will provide the basis for the creation of new means for increasing the general resistance of animals, the prevention and treatment of metabolic diseases, as well as current schemes of their application in the context of industrial livestock management (Sakhniuk, 2008; Bezukh, Chub and Nadtochii, 2011).

Laboratory markers of metabolic disorders in cows comprise a small number of biochemical parameters, which include the determination of various parameters reflecting the state of protein, carbohydrate, lipid and mineral metabolism, as well as the activity of some key serum enzymes. Evaluation of the results of biochemical blood testing, especially in the dynamics — in the process of disease development, has great diagnostic and prognostic value in many acute and chronic diseases of the internal organs.

**The purpose of the research** was to determine the characteristics of metabolic disorders in dairy cows in the dry periods, calving, and after calving periods in FG 'Mriia' in Rivne district of Rivne region.

**Materials and methods.** Researches were conducted in FG 'Mriia' (v. Velyka Omeliana of Rivne district, Rivne region) in cows of Ukrainian black-rumped dairy breed, aged 4–6 years, live weight 470–500 kg, productivity of 4,700–5,500 kg of milk per lactation, which were kept in the stall-pasture system.

Clinical examination of cattle (examination, thermometry, palpation) was performed by conventional methods (Levchenko et al., 2012).

The presence of metabolic changes in the body of cows in dry period (n = 6), post-partum cows (n = 8), and cows 10 days after calving (n = 8) were determined by

biochemical parameters (markers) in blood serum samples, which were tested by conventional biochemical methods (Pokrovskiy, 1969; Antonov, Fedotova and Sukhaya, 1989) using the biochemical analyzer 'Cobas c 311' (Roche Diagnostics, Switzerland) and the content of inorganic elements was determined using the atomic absorption spectrophotometer 'C-115M1' in the Laboratory of Experimental and Analytical Methods of the Research Epizootology Station of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine (Rivne, Ukraine).

Statistical processing of the results was performed by variation statistics using Statistica 6.0 (StatSoft, USA). Nonparametric research methods were used (Wilcoxon-Mann-Whitney test). The arithmetic mean (x), standard error of the mean (SE) was determined. The difference between the two averages was considered statistically significant when: * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$.

**Results and discussion.** According to the results of obstetric examination of sick animals, carried out in the dry season, such diseases as ketosis (8.9%), fatty liver syndrome (6.7%), and udder edema (5.6%) were established. In cows, the level of ketone bodies was 2.0 ± 0.04 mmol/l. 75.0% of animals diagnosed with ketosis in the dry period were prone to develop other diseases such as ovarian cysts and postpartum endometritis. Idiopathic diseases averaged 12.2%. Among the concomitant pathologies, the detention of litter was detected — 32.2%. 25 cases (27.8%) of post-partum paresis were recorded out of 90 cows examined. During the analysis of clinical symptoms of post-partum paresis it was determined that cows had decreased appetite, reduced acts of urination and defecation; cows became weak, sluggish, disorders of movement coordination were observed, the body temperature was decreased (n = 15) within 35.4 ± 1.01 to 37.6 ± 1.13°C.

The analysis of the results of primary biochemical studies of cattle blood revealed that in the serum of cows in all physiological groups, the values of protein, hydrocarbon and fat metabolism, namely total protein, albumin, glucose, and total cholesterol were lower relative to the average levels (Motuzko, Nikitin and Gusakov, 2008). In the body of cows, the content of total globulins was reduced by 16.6% during the dry period. A similar trend is observed with the content of vitamin A, total calcium, and inorganic phosphorus.

In cows, on the 10$^{th}$ day after calving, the albumin content was reduced by 18.8% ($p < 0.01$) relative to the lower reference level. In addition, in post-partum cows, a decrease in the content of one of the most sensitive markers — total cholesterol by 13.5% ($p < 0.05$), and glucose content by 26.4% ($p < 0.05$) relative to the lower reference level (Table 1).

**Table 1** — Biochemical parameters of blood of cows with different physiological state (M ± m)

| Biochemical parameters | Serum of animals of different physiological groups | | | Reference level, adult animals (Motuzko, Nikitin and Gusakov, 2008) |
|---|---|---|---|---|
| | cows in dry period (n = 6) | cows after calving (n = 8) | cows 10 days after calving (n = 8) | |
| *Indicators of protein metabolism* | | | | |
| Total protein, g/l | 58.49 ± 1.53 | 67.28 ± 2.2* | 68.19 ± 0.96 | 72.0–86.0 |
| Albumin, g/l | 37.70 ± 0.7 | 29.66 ± 0.59** | 22.51 ± 0.92 | 27.5–39.4 |
| Total globulins, g/l | 24.14 ± 1.63 | 39.25 ± 2.51 | 46.29 ± 0.75 | 28.9–48.6 |
| Urea, mmol/l | 4.60 ± 0.42 | 5.34 ± 0.18 | 4.96 ± 0.26 | 3.5–6.0 |
| Creatinine, μmol/l | 99.65 ± 5.80 | 111.38 ± 4.07 | 121.86 ± 4.09 | 80.0–130.0 |
| *Carbohydrate metabolism rate* | | | | |
| Glucose, mmol/l | 2.15 ± 0.09 | 1.84 ± 0.06* | 2.71 ± 0.12 | 2.5–3.5 |
| *The index of fat metabolism* | | | | |
| Total cholesterol, mmol/l | 3.22 ± 0.31 | 1.99 ± 0.07* | 2.50 ± 0.08 | 2.3–4.5 |
| *The activity of hepatospecific enzymes* | | | | |
| ALT, mmol/h×l | 0.55 ± 0.018 | 1.13 ± 0.019 | 1.21 ± 0.11 | 0.6–1.8 |
| AST, mmol/h×l | 1.73 ± 0.24 | 2.36 ± 0.16 | 2.41 ± 0.08 | 0.6–3.0 |
| *Indicators of vitamins and macronutrients* | | | | |
| Vitamin A, μg% | 13.53 ± 1.14*** | 10.63 ± 0.93** | 20.56 ± 0.36 | Not less 25 |
| Vitamin E, μg/ml | 3.56 ± 0.19 | 2.81 ± 0.1* | 5.0 ± 0.24 | 4.0–6.0 |
| Total calcium, mmol/l | 1.92 ± 0.03*** | 2.06 ± 0.07 | 2.76 ± 0.16 | 2.25–3.0 |
| Inorganic phosphorus, mmol/l | 1.32 ± 0.08 | 1.3 ± 0.13 | 1.03 ± 0.06 | 1.45–2.10 |
| The ratio, Ca:$P_n$ | 1.8 ± 0.06** | 2.71 ± 0.07 | 0.66 ± 0.09 | 1.43–1.55 |

Notes: * — $p < 0.05$; ** — $p < 0.01$; ** — $p < 0.001$ relative to the lower reference level.

According to the results of studies of inorganic elements in blood sera it has been established:

— in the group of cows in dry period — lack of zinc (7.8%), copper (18.6%), manganese (11.8%), selenium (50.9%), iodine (33.9%), and cobalt (26.8%) relative to the lower reference level and excess of iron (1.8 times) and nickel (0.9%) relative to the upper reference level. Due to the low content of total calcium, inorganic phosphorus, manganese cows are at high risk of postpartum paresis. The low content of total calcium, copper, iodine, and selenium poses risks of delayed litter and low content of cobalt — the risk of ketosis. Deficiency of total calcium, inorganic phosphorus, manganese, selenium, vitamins A and E during the dry season results in abortions and stillbirths;

— in the post-partum group, there was a shortage of zinc (4.95%), copper (3.3%), selenium (36.7%), and iodine (39.7%) relative to the lower reference level and excess of iron (1.8 times) relative to the upper reference level. In this physiological period, the lack of zinc, copper, selenium, vitamins A and E is one of the reasons for the decrease in the humoral level of the immune system, which creates a basis for the activation of conditionally pathogenic microflora and the development of postpartum sepsis;

— lack of zinc (12.4%), manganese (6.0%), selenium (29.2%), and cobalt (27.6%) in cows 10 days after calving is one of the reasons of delayed heat period and low fertility, followed by early embryonic mortality (Table 2).

As a result of the conducted studies and in accordance to 'Nutrient Requirements of Dairy Cattle' (NRC, 2001), the farm was recommended:

— to investigate the nutritional value of all feed used in cattle feeding to eliminate dietary imbalances, especially in protein, carbohydrate, lipid and mineral constituents;

— for correction of the lack of microelements in animals, it is necessary to enter in the rations of cattle preparations of zinc (zinc sulfate 60–70 mg/animal/day) for 7–14 days (further diet of animals should contain zinc in the amount of 30.0 mg/kg of dry matter) and manganese (manganese sulfate or chloride) at the rate of 40.0 mg of the element per 1 kg of dry matter; selenium preparations (the content of the element should be 0.1–0.3 mg/kg of dry matter);

— to introduce into the diet (or inject) vitamins A and E in therapeutic doses according to the instructions for use. A detailed correction of cattle mineral nutrition can also be achieved by introducing a blend containing the above trace elements, after examining their actual content in the diet;

— to examine the feed (especially mineral supplements) and water for their content to detect the source of the excess of iron;

— for timely identification of problematic points in the state of metabolism in the body of cattle, periodically (in spring and autumn) to examine blood samples from all age groups of animals (especially calves up to the age of 1 month, heifers, before first fertilization, cows, before changing conditions of keeping) content of glucose, total cholesterol, total protein, albumin, urea, creatinine, activity of indicator hepatospecific enzymes, content of vitamins A and E, content of inorganic elements.

**Table 2 —** Content of microelements in serum of cows in different physiological groups (M ± m)

| Element | Different physiological groups | | | Reference level, adult animals (Motuzko, Nikitin and Gusakov, 2008) |
|---|---|---|---|---|
| | Cows in dry period (n = 6) | Cows after calving (n = 8) | Cows 10 days after calving (n = 8) | |
| Zinc, μg% | 92.18 ± 0.88 | 95.06 ± 1.44 | 87.63 ± 1.24 | 100.00–220.0 |
| Copper, μg% | 65.13 ± 1.28 | 77.33 ± 1.15 | 114.16 ± 2.1 | 80.00–120.00 |
| Iron, μg% | 385.35 ± 11.41 | 200.41 ± 3.33 | 194.15 ± 16.15 | 85.00–210.00 |
| Manganese, μg% | 3.53 ± 0.19 | 4.16 ± 0.21 | 3.76 ± 0.09 | 4.00–6.00 |
| Selenium, μg% | 3.68 ± 0.38 | 4.75 ± 0.12 | 5.31 ± 0.21 | 7.50–10.00 |
| Lead, μg% | Not found | Not found | Not found | – |
| Nickel, μg% | 5.45 ± 0.068 | 5.15 ± 0.14 | 4.76 ± 0.19 | 2.80–5.40 |
| Iodine, nmol/l | 198.3 ± 0.11 | 181.1 ± 3.21 | 309.25 ± 2.66 | 300–500 |
| Cobalt, μg% | 1.83 ± 0.037 | 2.4 ± 0.009 | 1.81 ± 0.16 | 2.50–5.00 |

**Conclusions.** 1. The following metabolic disorders have been established by biochemical markers in animals of the respective groups, namely: in cows in the dry period — the decrease of the total protein level along with its globulin fraction, the decrease of glucose content relative to the reference values of these indicators; of cows in the calving group on the background of changes in the proteinogram — a decrease in total cholesterol was determined; and in cows 10 days after calving — a decrease in the content of albumin relative to the reference values of these indicators was determined.

2. According to the results of determination of the content of inorganic elements, it is established that in the body of cattle there is: in the group of dry cows — lack of zinc, copper, manganese, selenium, iodine, and cobalt and excess of iron and nickel; in the group of calving cows,

there is a lack of zinc, copper, selenium, and iodine and excess of iron; in the group of cows 10 days after calving — lack of zinc, manganese, selenium, and cobalt.

3. The data obtained can be interpreted to diagnose metabolic disorders in other farms and to take corrective action.

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# BIOCHEMICAL PARAMETERS OF BLOOD SERA OF SHEEP VACCINATED AGAINST CONTAGIOUS AGALACTIA

**Stegniy B. T. [1], Bohach D. M. [1], Vovk D. V. [1], Bohach M. V. [2]**

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**Summary.** The paper presents data on the positive effect of the inactivated vaccine against contagious agalactia of sheep and goats developed in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine) on the biochemical and immunological parameters of sheep blood serum. It has been proved that the vaccine is not reactogenic, does not have immunosuppressive action and corrects the recovery of serum albumin concentration in vaccinated sheep, namely by reducing α-globulins by 28.5% and β-globulins by 36.8% and has a positive effect on the growth of γ-globulin by 31.5%, activating the humoral level of immunity

**Keywords:** contagious agalactia, vaccine, biochemical parameters, albumins, globulins

**Introduction.** Improvement of existing and development of new means for the specific prevention of infectious diseases is an extremely important task for veterinary science. No less important is finding ways to use vaccines against pathogenic agents from local strains and creating inactivated vaccines. The advantage of such vaccines is the creation of immunity in animals to diseases in the short term, depending on the epizootic situation in the farm, district or region (Ryzhenko et al., 2010; Kosenko and Liubenko, 2001).

In Ukraine, contagious agalactia is recorded only in some districts of the Odesa region, but due to the active development of the sheep industry, the disease can spread to other regions. That is why the directions for studying of epizooty and the development of specific means for prevention are relevant (Stegniy et al., 2018).

Research and correction of the state of general resistance of animals will allow realizing potential, genetically conditioned possibilities of an organism (Perederiy et al., 1995).

The study of immunological parameters is a key point for the detection of immunodeficiency and immunopathological state, primary assessment of the immune state of the organism, as well as in the diagnosis, treatment and prognosis of the disease (Tymoshenko, Maslak and Maslak, 2009; Petrov and Lebedev, 1984).

The level of concentration of total immunoglobulins in the blood serum of animals is a criterion for evaluating the functional activity of the B-cell link of immune system, which allows to determine objectively the state of the immune status of animals (Kosenko et al., 2004).

**The purpose of the work** was to determine the effect of an inactivated vaccine against contagious agalactia of sheep and goats developed in NSC 'IECVM' on the biochemical and immunological parameters of sheep blood serum.

**Materials and methods.** The experimental part of the work was carried out in the conditions of a sheep farm PE 'Borlak' (v. Dmitrivka, Bolgrad district, Odesa region). Two groups of sheep (n = 15) were formed in the farm on the principle of analogues, for which the same conditions of feeding, care and keeping were provided.

In the experimental group, sheep were vaccinated with an inactivated vaccine against contagious agalactia of sheep and goats developed in the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM'). The vaccine was used twice subcutaneously in the tail fold at a dose of 1 $cm^3$ with an interval of 30 days.

Control of the vaccine effectiveness was carried out by biochemical and immunological parameters of the blood. For research, blood samples were collected from sheep before vaccination, on the $14^{th}$, $21^{st}$, and $30^{th}$ days after vaccination and on the $60^{th}$ day — revaccination.

Biochemical parameters of sheep blood serum reflecting the functional state of the liver were determined using an automatic biochemical analyzer 'IDEXX VestTest' ('IDEXX Laboratories', USA). Additionally, blood samples were tested for circulating immune complexes (Grinevich and Alferov, 1981) and seromucoids (Weimer and Moshin, 1953) in the Laboratory of Biochemistry of the NSC 'IECVM' (Kharkiv, Ukraine).

**Results and discussion.** The state of protein metabolism in the body of animals indicates the nature of biochemical processes that occur in the body under a certain antigenic load.

It was established that inactivated vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') influences and corrects protein metabolism in the body of vaccinated animals that is shown by the obtained results (Table).

**Table —** Biochemical parameters of blood sera of sheep inoculated with an inactivated vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') (n = 15, M ± m)

| Parameters | Research period, days | Groups | |
|---|---|---|---|
| | | experimental | control |
| Total protein, g/l | b/v | 72.18 ± 2.08 | 71.95 ± 2.06 |
| | 14 | 72.25 ± 1.04 | 70.21 ± 2.72 |
| | 21 | 72.98 ± 0.96 | 72.20 ± 1.09 |
| | 30 | 72.76 ± 2.12 | 71.39 ± 1.54 |
| | 60 | 73.31 ± 0.90 | 71.20 ± 2.13 |
| Albumins, g/l | b/v | 31.58 ± 2.21 | 31.14 ± 4.41 |
| | 14 | 33.15 ± 1.82 | 31.01 ± 2.17 |
| | 21 | 36.39 ± 0.75 | 32.22 ± 1.96 |
| | 30 | 36.91 ± 2.14 | 31.85 ± 2.23 |
| | 60 | 36.91 ± 2.09 | 32.29 ± 1.16 |
| α-globulins, g/l | b/v | 13.57 ± 1.12 | 13.98 ± 2.92 |
| | 14 | 11.96 ± 0.72 | 13.06 ± 1.73 |
| | 21 | 10.65 ± 2.17 | 13.72 ± 0.55 |
| | 30 | 9.95 ± 1.92 | 13.91 ± 2.17 |
| | 60 | 10.06 ± 0.34 | 13.09 ± 1.24 |
| β-globulins, g/l | b/v | 10.75 ± 0.56 | 10.92 ± 2.02 |
| | 14 | 10.16 ± 1.29 | 11.72 ± 0.98 |
| | 21 | 7.52 ± 0.48 | 11.39 ± 1.17 |
| | 30 | 7.22 ± 2.12 | 11.42 ± 2.62 |
| | 60 | 7.32 ± 0.19 | 11.05 ± 1.09 |
| γ-globulins, g/l | b/v | 16.28 ± 0.53 | 15.91 ± 2.01 |
| | 14 | 16.98 ± 1.81 | 15.02 ± 1.01 |
| | 21 | 18.42 ± 2.04 | 14.87 ± 0.94 |
| | 30 | 18.68 ± 0.16 | 14.21 ± 1.04 |
| | 60 | 19.02 ± 1.32 | 14.77 ± 2.07 |
| A/G ratio | b/v | 0.8 | 0.8 |
| | 14 | 0.9 | 0.8 |
| | 21 | 1.0 | 0.8 |
| | 30 | 1.0 | 0.8 |
| | 60 | 1.0 | 0.8 |
| CIC, mg/ml | b/v | 0.25 ± 0.03 | 0.25 ± 0.01 |
| | 14 | 0.26 ± 0.01 | 0.25 ± 0.01 |
| | 21 | 0.28 ± 0.02 | 0.24 ± 0.02 |
| | 30 | 0.29 ± 0.02 | 0.24 ± 0.02 |
| | 60 | 0.29 ± 0.01 | 0.24 ± 0.02 |
| Sero-mucoids, mg/ml | b/v | 0.26 ± 0.01 | 0.26 ± 0.01 |
| | 14 | 0.27 ± 0.02 | 0.26 ± 0.01 |
| | 21 | 0.26 ± 0.01 | 0.27 ± 0.02 |
| | 30 | 0.26 ± 0.01 | 0.27 ± 0.02 |
| | 60 | 0.26 ± 0.01 | 0.27 ± 0.02 |

Notes: b/v — before vaccination; * — $p < 0.05$, ** — $p < 0.01$, *** — $p < 0.001$ compared to the control group.

According to the biochemical parameters of sheep blood serum, the albumin fraction in the experimental and control groups was almost equal — 31.58 ± 2.21 and 31.14 ± 4.41 g/l, respectively. After vaccination with the vaccine in the experimental group on the 21st day, the content of albumin increased by 12.9% and amounted to 36.39 ± 0.75 against 32.22 ± 1.96 g/l in control. On the 30th day after vaccination, the indicator increased by 15.9% against control and was almost at this level — 36.91 ± 2.09 g/l until the 60th day of the experiment — after revaccination, which indicates stabilization of homeostasis of sheep organism.

According to the results of the studies, the quantitative content of globulin fractions of proteins, namely α-, β- and γ-globulins before vaccination in the experimental and control groups was almost at the same level with a difference of no more than 1.2%. In the experimental group on the 14th day after vaccination, the content of α-globulins decreased by 8.4% and amounted to 11.96 ± 0.72 against 13.06 ± 1.73 g/l in the control. On the 21st day, the indicator decreased by 22.4%, that is to 10.65 ± 2.17 against 13.72 ± 0.55 g/l in control. On the 30th day of the experiment, the lowest index of α-globulin content was reached — 9.95 ± 1.92 g/l, which is 28.5% less than in the control. Significantly decreased β-globulin content on the 21st day after vaccination by 34% and on the 30th day by 36.8%, that is 7.22 ± 2.12 against 11.42 ± 2.62 g/l in control. No significant fluctuations were observed after revaccination on the 60th day. The content of γ-globulins before vaccination in the experimental group of sheep was 16.28 ± 0.53 g/l, and in the control 15.91 ± 2.01 g/l. Significant increase of the indicator in the experimental group occurred on the 21st day, by 23.9%, compared with the control, and on the 30th day the content of γ-globulins increased by 31.5% and amounted to 18.68 ± 0.16 against 14.21 ± 1.04 g/l in control. After revaccination, on the 60th day the content of γ-globulins increased to 19.02 ± 1.32 against 14.77 ± 2.07 g/l in control, which is 28.8% more.

Based on the analysis of the results of the studies, it was found that hypergammaglobulinemia is the result of increased synthesis of immunoglobulins of all classes. During all immunological reactions, especially the γ-globulin fraction of the protein tends to increase, namely when vaccinated with an inactivated vaccine against contagious agalactia in sheep and goats. It is known that the bulk of the antibodies contain precisely γ-globulins, which provide humoral protection of the body. Increasing their number in the blood serum characterizes the morphological maturity and functional completeness of the body's immune reactive system.

The increase in albumin content on the 21st day after vaccination influenced the formation of A/G coefficient which in the experimental group was 1.0. It should be noted that in clinically healthy animals it is 0.8–1.0.

Certain immunosuppressive effect of the vaccine on the body of sheep was evaluated by the level of seromucoids. The preparation did not cause immunosuppressive effects on the body of sheep after vaccination and revaccination.

Seromucoid level in the test and control animals remained almost unchanged and was from 0.26 ± 0.01 to 0.27 ± 0.02 mg/ml during the study period.

The administration of the vaccine against contagious agalactia of sheep and goats (NSC 'IECVM') increases the content of total protein on the 30th day by 1.9%, and by 3% on the 60th day, and circulating immune complexes — by 20.8% on the 30th and 60th days.

**Conclusion.** The vaccine against contagious agalactia of sheep and goats is not reactogenic, does not have immunosuppressive action and corrects the recovery of albumin concentration in serum of vaccinated sheep, namely by reducing α-globulins by 28.5% and β-globulins by 36.8% and has a positive effect on increasing γ-globulins by 31.5%, activating the humoral level of immunity.

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# TREATMENT ACTIVITIES IN MALES WITH GONADODYSTROPHY USING DRUGS BASED ON NANOBIOMATERIALS

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**Summary.** The article presents data on the development of innovative methods of treating males with gonadodystrophy using drugs based of nanobiomaterials. The complex drug 'Karafand+OV,Zn' contains carotenoids, phytoandrogens, and nanomaterials — nanoparticles of gadolinium orthovanadate activated by europium and zinc carbonate, it restores and activates the function of the testes, which generally normalizes the reproductive function of males. The developed methods are simple to implement and can be introduced into the practice of veterinary medicine as correctors of vitamin-hormonal metabolism, with a predominant antioxidant effect

**Keywords:** andrology, males, gonadodystrophy, therapy, nanomaterials, complex preparation 'Karafand+OV,Zn'

**Introduction.** Alimentary deficient and toxic factors are a common cause of the development of various pathological processes, including processes in the reproductive system of males. Such processes cause damage to the tissues of regulatory organs and organs performing the reproductive function, mainly of a dystrophic nature (Medvedev and Turchanov, 1999; Naumenko, 2009; Koshevoy and Naumenko, 2015).

The combination of such processes with a marked decrease in the reproductive function of males, without clinical manifestations, is called gonadodystrophy. Depending on the etiological factor, there are distinguished an alimentary type of gonadodystrophy (in case of alimentary deficient states in the body — a deficiency of carotene, vitamin A, Zinc) and toxic (with nitrate-nitrite toxicosis, mycotoxicosis) (Koshevoy et al., 2015; Naumenko and Koshevoy, 2017).

The development of methods for the treatment and prevention of gonadodystrophy in males is an urgent problem that requires constant research; the creation of drugs based on nanobiomaterials is its main component (Koshevoy et al., 2016).

**The purpose of the study** was to develop innovative methods for treating males with gonadodystrophy using drugs based on nanobiomaterials and to determine the therapeutic efficacy and pharmacological activity of the complex drug 'Karafand + OV, Zn'.

**Materials and methods.** This work was performed in the laboratories of the Department of Veterinary Reproductology of the Kharkiv State Zooveterinary Academy and in the Laboratory of Nanostructured Organic Materials of the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine (Kharkiv, Ukraine).

The studies were carried out on bulls (n = 8) and boars (n = 12) with alimentary type gonadodystrophy, which belonged to the Training and Production Center for Plant Breeding and Livestock of the Kharkiv State Zooveterinary Academy, the Institute of Animal Breeding of the National Academy of Agrarian Sciences of Ukraine (Kharkiv, Ukraine), and the farms of Kharkiv, Dnipropetrovsk and Zaporizhzhya regions of different forms of ownership.

For the treatment of males with gonadodystrophy, the 'Karafand+OV,Zn' complex drug was used, which contained carotenoids, phytoandrogens, and nanomaterials — gadolinium orthovanadate activated by europium and zinc carbonate, in recommended doses (Koshevoy et al., 2017a).

For diagnosis, we used the clinical remotely-contactless and non-invasive method developed by us (Koshevoy et al., 2017b); biochemical and hormonal research methods were used to determine therapeutic efficacy. Blood was taken for studies at the beginning of the experiment (control) and on the 20$^{th}$ day after giving the drug.

Biochemical blood test was performed in the Central Research Laboratory of the National University of Pharmacy (Kharkiv, Ukraine). The amount of zinc was determined by atomic absorption spectrophotometry. Chemiluminescent analysis was performed in Laboratory of Nanostructured Organic Materials of the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine. The concentration of testosterone was determined in the V. Danilevsky Institute for Endocrine Pathology Problems of the National Academy of Medical Sciences of Ukraine (Kharkiv, Ukraine) using the ELISA test system ('Granum Laboratory' Ltd., Ukraine).

**Results.** We have developed an innovative method of treating males with gonadodystrophy using the complex drug 'Karafand+OV,Zn'. This drug was administered, according to the recommendations, once a day, orally, the dosage was adjusted by the data on the number of deficient factors in feed and water.

As can be seen from the data in the Table the drug showed high therapeutic efficacy.

**Table —** Biochemical blood parameters of males under the influence of the complex drug 'Kararafand+OV,Zn' (M ± m)

| Indicators | Bulls (n = 8) | | Boars (n = 12) | |
|---|---|---|---|---|
| | before administration | after administration | before administration | after administration |
| Vitamin A, μmol/l | 0.8 ± 0.06 | 1.4 ± 0.08* | 0.19 ± 0.03 | 0.62 ± 0.05* |
| Carotene, μmol/l | 2.7 ± 0.02 | 7.9 ± 0.05 | not determined | not determined |
| Zinc, μmol/l | 12.4 ± 0.21 | 20.2 ± 0.14 | 8.4 ± 0.02 | 11.6 ± 0.23* |
| Total protein, g/l | 72.5 ± 0.4 | 82.2 ± 0.3* | 68 ± 2.2 | 81 ± 1.4* |
| Inorganic calcium, μmol/l | 2.79 ± 0.01 | 2.96 ± 0.01* | 2.5 ± 0.13 | 3.5 ± 0.16* |
| Inorganic phosphorus, μmol/l | 1.5 ± 0.01 | 2.2 ± 0.01 | 1.32 ± 0.006 | 1.98 ± 0.06* |
| ***Content in red blood cells:*** Malonic dialdehyde, μmol/l | 45.7 ± 0.04 | 24.6 ± 0.05 | 57.3 ± 0.03 | 35.3 ± 0.01 |
| Catalase, μmol/$H_2O_2$/l-min | 15.9 ± 0.06 | 26.7 ± 0.08 | 14.8 ± 0.02 | 37.8 ± 0.4 |
| Reduced glutathione, μmol/l | 3.24 ± 0.04 | 3.87 ± 0.07* | 3.21 ± 0.88 | 3.81 ± 0.07 |
| ***Serum contents:*** Malonic dialdehyde, μmol/l | 0.82 ± 0.04 | 0.28 ± 0.05* | 0.83 ± 0.03 | 0.41 ± 0.01* |
| Catalase, μmol/$H_2O_2$/l-min | 20.3 ± 0.07 | 43.2 ± 0.04 | 23.7 ± 0.72 | 41.4 ± 0.52* |
| Superoxide dismutase, st. un./mgHb | 5.3 ± 0.09 | 9.3 ± 0.07 | 5.7 ± 0.41 | 10.7 ± 0.02* |
| Prooxidant-antioxidant ratio | 3:1 | 1:1 | 3:1 | 1:1 |
| ***Chemiluminescence:*** Light sum, un. | 8.4 ± 0.04 | 3.5 ± 0.05 | 8.1 ± 0.12 | 4.2 ± 0.1* |
| Red blood cells, $\times 10^{12}$/l | 5.9 ± 0.1 | 7.2 ± 0.3* | 5.8 ± 0.7 | 6.8 ± 0.6 |
| Hemoglobin concentration, g/l | 98 ± 0.03 | 114 ± 0.02 | 86 ± 0.01 | 104 ± 0.02 |
| Concentration of 2,3-diphosphoglycerate, mmol/l | 0.8 ± 0.031 | 1.3 ± 0.031* | 0.9 ± 0.045 | 1.4 ± 0.049* |
| Testosterone concentration | 7.1 ± 0.12 mmol/l | 16.9 ± 0.21 mmol/l | 4.9 ± 0.35 nmol/l | 17.7 ± 0.02 nmol/l |

Note: * — $P < 0.001$ in comparison with the indicators before drug administration.

The drug showed the greatest efficiency in terms of the content of environmentally deficient factors: carotene concentration in bulls increased by 1.92 times; vitamin A content increased in bulls by 0.75 times, in boars — by 2.26 times; zinc content increased in bulls by 62.9%, in boars — by 38.1%. Normalization of protein-mineral metabolism was noted.

Positive dynamics was found in the prooxidant-antioxidant system and oxygen metabolism: the content of malonic dialdehyde in blood serum and erythrocytes decreased in bulls — by 65 and 46%, in boars — by 51 and 38.4%, respectively; catalase activity increased significantly and SOD in the blood serum of bulls by 112 and 75.5%, boars — by 87.2 and 87.7%, respectively; catalase and reduced glutathione increased in red blood cells of bulls by 62.9 and 19.4%, boars — 155 and 18.7%, respectively.

A significant increase in the concentration of 2,3-diphosphoglycerate was noted (in bulls — by 62.5%, in boars — by 55.6%).

By increasing the testosterone content in the blood serum of males (in bulls — by 1.4 times, in boars — by 2.6 times), we can conclude about positive changes in hormonal levels.

**Conclusions.** Innovative methods of treating males with gonadodystrophy using drugs based on nanobiomaterials are effective.

The complex preparation 'Karafand+OV,Zn' restores and activates the function of the testes, which generally normalizes the reproductive function of males.

This drug and its application are simple and can be successfully implemented in the practice of veterinary medicine.

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# PARASITES OF PANTHER CHAMELEONS (*FURCIFER PARDALIS*) GROWN IN CAPTIVITY AND BROUGHT FROM THE WILD

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**Summary.** Reptile parasites imported from the wild differ from those grown in captivity. Thus, captive-grown reptiles tolerate the process of disadaptation better than imported wild animals, even under proper conditions of keeping and feeding. It should be noted that determining the origin of reptiles is sometimes difficult or impossible. For this, special methods are needed. In this regard, the purpose of research was to confirm or refute the theory, in reptiles from different places of residence, various parasites are found. We studied panther chameleons (*Furcifer pardalis*) imported from the wild and raised in captivity. To determine the parasites in the laboratory, methods of native smear, sequential washing and flotation were used. 10 species of intestinal parasites were found in panther chameleons imported from the wild, in particular Trematoda gen. sp. 1, *Tremaitoda* gen. sp. 2, Cestoda gen. sp., *Spinicauda freitasi* (Olfers, 1919), *Hexametra angusticaecoides* (Chabaud et Brygoo, 1960), Pharyngodonidae gen. sp., spirurates of the genus *Thubunaea* sp., larvae of the family Rhabdiasidae gen. sp., flagellates from the series Kinetoplastida gen. sp. and *Eimeria* sp, with prevalence 87.56%. In panther chameleons grown in captivity only Pharyngodonidae gen. sp. was found, prevalence was 94.05%. It is noted that under appropriate conditions of keeping and feeding in captive panther chameleons, a small number of parasites with a direct development cycle and their insignificant toxic effect on the body can develop

**Keywords:** intestinal parasites, panther chameleons, prevalence, invasion

**Introduction.** Reptiles are becoming more common in zoos and private collections. The incredible variety of them makes it possible to create terrariums with different biotopes. Some reptiles need arid terrariums with sand or, conversely, moist, dense vegetation, as well as rocky and water-filled bottoms. However, the problem is that not all reptiles can be purchased at pet stores or specialty nurseries (Vasil'ev, 2005).

Many species of reptiles are brought from the wildlife. Reptiles imported from the wild still have many different ailments. Most of these diseases are chronic. At the same time, chronic illnesses are exacerbated by the stress that occurs during trapping and transportation. Up to 60% of reptiles died during disadaptation (Cowan, 1980).

In Slovenia Rataj et al. (2011) found *Hexametra angusticaecoides* ascarids in Yemeni chameleons (*Chamaeleo calyptratus* Duméril et Bibron, 1851). In addition, these studies indicate a high infestation of snake parasites from the wild, in particular prevalence was 47.3%.

In Poland Okulewicz et al. (2015) also found *Parapharyngodon* sp., *Pharyngodon* sp., *Eimeria* sp., *Isospora* sp., *Nyctotherus* sp., *Balantidium* sp.

In the reptiles of the Kiev Zoo infection with nematodes (ascarids and oxyurises) was also observed, in particular in bearded agamas (*Pogona barbata*) with prevalence 53.6% (Dashchenko and Semenko, 2017).

It should also be noted that the capture of reptiles from the wild can also affect the ecology of the area, lead to population decline and even the extinction of the species itself. This is why it is forbidden in some countries to export endemic animals (Stoyanov and Stoyanova, 2018).

Reptiles that have been bred in captivity under appropriate conditions of retention have significantly fewer infectious diseases. Nor do they affect the number of animals in the wild. For sales, even abroad, it is legally easier to draw up captive reptile documents. In general, captive reptile farming is more profitable than catching, transporting and quarantining wild reptiles (Jacobson, 2007).

Most terrarium holders still want to get an animal that was bred in captivity. At the same time, there are not enough techniques to check the origin of reptiles. Parasitological research methods come to the rescue. It should be noted that the retention of reptiles in terrariums and the feeding of specially grown fodder facilities eliminates the possibility of transmitting a number of pathogens. Therefore, using parasitological studies can determine the origin of certain reptiles (Vasil'ev, 2005).

**The purpose of the study** is to confirm or refute the theory that different parasites are found in reptiles from different locations.

**Material and methods.** The studies were performed in the laboratory of the Department of Parasitology and Tropical Veterinary Medicine on Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine) during 2016–2018. Fecal samples from panther chameleons (*Furcifer pardalis* Cuvier, 1829) were used. The reptiles were kept at the Nature Center 'Bion' (Kyiv, Ukraine).

Fecal samples were collected with tweezers, which were washed and disinfected in 70% alcohol after each sample was taken. The feces were placed in a disposable plastic

bag, signed and logged in to register primary studies. The test material was transported to the study site in a cold bag with a temperature of 4–9 °C. The studies were carried out on the day of fecal sampling and not later than three hours after their selection (Tret'yakov, Yevdokimov and Shabaev, 2006; Zajac and Conboy, 2012).

Each sample of reptile feces was investigated using the methods of native smear, sedimentation and flotation according to Fülleborn (Kotel'nikov, 1983). Data from three studies were summarized and analyzed. The identification of eggs, larvae and oocysts of the parasites was performed using atlases of Jacobson (2007), Vasil'ev (2005), Stoyanov and Stoyanova (2018).

646 panther chameleons were examined, among those 410 were imported from the wild and 236 were captive-bred, aged from 4 to 10 months (4–6 months — 152 and 6–10 months — 84 chameleons). 1938 laboratory tests were conducted.

**Results and discussions.** In the panther chameleons imported from the wild, 10 species of intestinal parasites were recorded, including two species of trematodes (Trematoda gen. sp. 1 and Trematoda gen. sp. 2), one species of cestodes (Cestoda gen. sp.), five nematode species (*Spinicauda freitasi* (Olfers, 1919), *Hexametra angusticaecoides* (Chabaud et Brygoo, 1960), Pharyngodonidae gen. sp., *Thubunaea* sp., and larvae Rhabdiasidae gen. sp.), flagellates from the series Kinetoplastida gen. sp. and oocysts of *Eimeria* sp.

According to the research, prevalence of invasion was 87.56%. The prevalence by various species of parasites in panther chameleons imported from the wild is shown in the Table.

**Table —** Prevalence of invasion in panther chameleons imported from the wild

| Parasites | Prevalence, % |
|---|---|
| Trematoda gen. sp. 1 | 12.93 |
| Trematoda gen. sp. 2 | 44.15 |
| Cestoda gen. sp. | 5.12 |
| *Spinicauda freitasi* | 35.12 |
| *Hexametra angustiaecoides* | 16.34 |
| Pharyngodonidae gen. sp. | 19.02 |
| *Thubunaea* sp. | 2.19 |
| Rhabdiasidae gen. sp. | 6.59 |
| Kinetoplastida gen. sp. | 18.78 |
| *Eimeria* sp. | 28.54 |

The most commonly recorded eggs were Trematoda gen. sp. 2. Slightly less eggs of *S. freitasi* and oocysts of *Eimeria* sp. were found. Eggs of Pharyngodonidae gen. sp. met less frequently. Very few flagellates Kinetoplastida gen. sp., *H. angustiaecoides* and Trematoda gen. sp. 1 were recorded. The larvae of the family Rhabdiasidae gen. sp., eggs of cestodes and spirurata of the genus *Thubunaea* sp. were rarely noted.

The causative agents of the intestinal invasion of the panther chameleons grown in captivity were only Pharyngodonidae gen. sp.

The prevalence of invasion by oxyurises in panther chameleons grown in captivity in the age group of 4–6 months is 68.42%, and in the age group of 6–10 months — 94.05%. Research shows that with age, the prevalence of invasion of panther chameleons grown in captivity increases.

The data obtained show that the prevalence of invasion in reptiles imported from the wild is lower than in those raised in captivity. Thus, in panther chameleons imported from the wild, it is 87.56%, in those grown in captivity — 94.05% for 6–10 months of their life. It should be noted that the toxic effect of oxygenates on the body panther chameleons is considered insignificant. At the same time, the pathogenic effect of the detected trematodes, nematodes and protozoa is much stronger (Vasil'ev, 2005).

It should also be noted that the prevalence of the invasion by the oxyurises of Pharyngodonidae gen. sp. reptiles imported from the wild are much lower (19.02%) than those grown in captivity (87.56%). This can be explained by the competition of parasites among themselves for the space and resources of the host organism (Jacobson, 2007).

**Conclusions.** Studies have shown that panther chameleons (*Furcifer pardalis*) imported from the wild are most often affected by Trematoda gen. sp. 2 (44.15%), *Spinicauda freitasi* (35.12%), *Eimeria* sp. (28.54%); slightly less oxyurises Pharyngodonidae gen. sp. (19.02%), flagellates Kinetoplastida gen. sp. (18.78%), *Hexametra angusticaecoides* (16.34%), Trematoda gen. sp. 1 (12.93%), Rhabdiasidae gen. sp. (6.59%), Cestoda gen. sp. (5.12%) and a little — *Thubunaea* sp. (2.19%). While panther chameleons, raised in captivity, are infected only with Pharyngodonidae gen. sp.

Therefore, under appropriate conditions of keeping and feeding, panther chameleons grown in captivity are infected by nematodes with a simple cycle of development and those that do not have a significant negative impact on the body. Parasites with a complex developmental cycle cannot infect these reptiles in a closed terrarium system. Therefore, researching reptiles on parasitic diseases can determine their origin.

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# Part 2. Biotechnology and genetics

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## ULTRASTRUCTURE AND BIOLOGICAL PROPERTIES OF AVIAN INFLUENZA VIRUSES FOLLOWING CRYOPRESERVATION

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**Summary.** Ultrastructure and infectious activity of avian influenza virus (strain A/Chicken/Sivash/02/05 (H5N1)) following cryopreservation and low temperature storage at –20, –70, and –196°C during various terms from 25 days up to 143 months using electron microscopy, serological and virological methods were investigated. Avian influenza viruses strain A/Chicken/Sivash/02/05 (H5N1) is stored in the Collection of Pathogens of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine), which was granted the National Endowment of Ukraine status. The conducted study allowed to reveal on electronograms the ultrastructural changes in AIV during long term storage (18 months) at moderately low temperature (–20°C), in particular loss of glycoprotein of peplomers in the majority of virions. The changes in ultrastructure of the virus samples were accompanied by a loss of hemagglutinating activity during long-term storage of AIV samples at moderately low temperature of –20°C. When storing the AIV samples at –70 and –196°C the virions generally remain negatively contrasted, keep peplomers for the studied storage duration

**Keywords:** avian influenza virus, morphology, ultrastructure, virion, cryopreservation, cryobank, cryodamage

**Introduction.** Avian influenza viruses strains isolated during infectious disease outbreaks from the brain of chickens, in the AR Crimea in 2005, are stored at the Cryobank of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' for further investigation of their biological properties, patenting, development of the preventive measures and therapy of viral infections in birds (Stegniy B., 2012; Stegniy M., 2006). Avian influenza viruses are the part of the Collection of Pathogens of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine', which was granted the National Endowment of Ukraine status.

As classified by the International Committee on Taxonomy of Viruses (Van Regenmortel et al, 2000) this virus belongs to the family Orthomyxoviridae, genus *Influenza virus A;* affects mainly birds of all ages. Humans are also susceptive to the avian influenza virus (AIV). Avian influenza is the most dangerous disease of birds, mammals and humans. Herewith the disease proceeds with the symptoms of injury of upper respiratory tract. In birds, the avian influenza virus causes respiratory and intestinal syndromes (Stegniy B., 2012).

High mortality due to avian influenza is observed in birds of about from 10 to 100%. Respiratory syndrome manifests itself in birds by a cough, conjunctivitis, rhinitis and sinusitis, hyperemia of mucous membranes and edema. Thus, from the foregoing it can be concluded that the avian influenza viruses play an important role in infectious pathology of humans and animals and are capable of overcoming the species barrier.

Avian influenza viruses possess a lipoprotein envelope, viruses bind to the receptors containing the sialic acid and exhibit a neuraminidase activity (Stegniy B., 2012; Stegniy M., 2006). The features of the family Orthomyxoviridae are: average size of virions from 80 to 150 nm; the presence of RNA; a membrane comprising the lipids; may occur filamentous form of virions.

**The aim of the work** was to assess the disorders in structural integrity of the virions, in particular viral shells and nucleocapsid during their low temperature storage in the Collection of pathogens.

**Materials and methods.** The research object was the AIV strain A/Chicken/Sivash/02/05 (H5N1), obtained from the collection of the Department of Avian Viral Diseases of the the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Freezing and storage of the collection samples of extra-embryonic virus-containing liquid was performed in a domestic refrigerator (–20 ± 0.1°C), low-temperature freezer (–70 ± 0.4°C) for various time periods: from 25 days and up to 143 months. Viruses are known to be generally more resistant to environmental and cryopreservation factors, therefore in many laboratories and at bioindustrial enterprises for storage of viruses there are used moderately low temperatures (–20 … –22°C).

This stipulated the choice of the range of cryopreservation and storage regimens of viral material.

Virus-containing liquid was packed into 1.8 and 2.0, 4.5 $cm^3$ cryovials ('Nunc', Denmark). Cooling of biological samples was performed using a refrigerator chamber; the samples were cooled passively in the refrigerator chamber. Temperature conditions were monitored by thermo gauge placed into the cooled object, or by low-temperature thermometer. The samples were either slowly thawed on air or in a water bath at 38–39°C for 1–2 min.

Titers of AIV infectious activity were determined by titration in 9–10-day-old chicken embryos, the embryonic virus infecting dose ($EID_{50}$/ml) was calculated according to the standard procedure (Stegniy B., 2012; Stegniy M., 2006). AIV hemagglutination titers were studied in hemagglutination test. The effect of low temperatures and deep cooling on the AIV viral ultrastructure was investigated using electron microscopy. The samples were inactivated with 0.5% formaldehyde solution.

The inactivation completeness was tested by means of three successive passages in 9-day-old chicken embryos. The virus samples were used for the electron microscopy studies if there was no infectious activity. The neutralization reaction (NR) in chicken embryos and assessment of antigen specificity of the AIV virus after cryopreservation was performed with different virus dilutions and constant dose of a specific serum. Once infected with a mixture of serum and virus the 8–10-day-old chicken embryos were incubated at 37°C for 8 days with daily ovoscopy.

The embryo death in the first 24 h was not taken into account because considered as non-specific. Neutralization was considered positive when no death of embryos was found as well as no changes characteristic for AIV resulting from neutralization of virus virulence by antibodies. The results were evaluated by the neutralization index: if higher than 2 lg — the reaction was considered as positive; 1.5–2 lg — doubtful; 1 lg — negative.

Electron microscopy was performed in the samples which were stored from 25 days up to 143 months at moderately low (–20°C), low (–70°C) and liquid nitrogen temperatures (–196°C). The copper grid platforms covered with formvar film were used. Adsorption of virus particles, preliminary purified in sucrose density gradient on the film substrate was carried out within 3–5 min. The resulted specimens were contrasted using phosphotungstic acid (Turcu et al., 1994; Ponomarev and Mishchenko, 2005; Ponomarev, 2001). Studies were performed with 'PEM-125' electron microscope using the 60–100 thousand times magnification at the premises of Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine.

Performing statistical analysis of the research results we calculated mean and standard deviation. Significance of differences between samples was assessed according to the Student-Fisher's test (Van Emden, 2019).

**Results and discussion.** Electron microscopic study of the post-thaw AIV samples, stored from 25 days at –20, –70, and –196°C to 143 months at –70 and –196°C demonstrated a moderate number of circular, elliptical and filamentous form virions with polymorphism and the sizes of 80–180 nm. Ultrastructure of AIV viruses' strain A/chicken/Sivash/02/05 (H5N1), was presented with a two-layer lipoprotein membrane with spicules of hemagglutinin and neuraminidase up to 12 nm and nucleocapsid of 90 nm diameter The central part of the circular virions from the mentioned samples was characterized by the concave presence (Fig. 1).

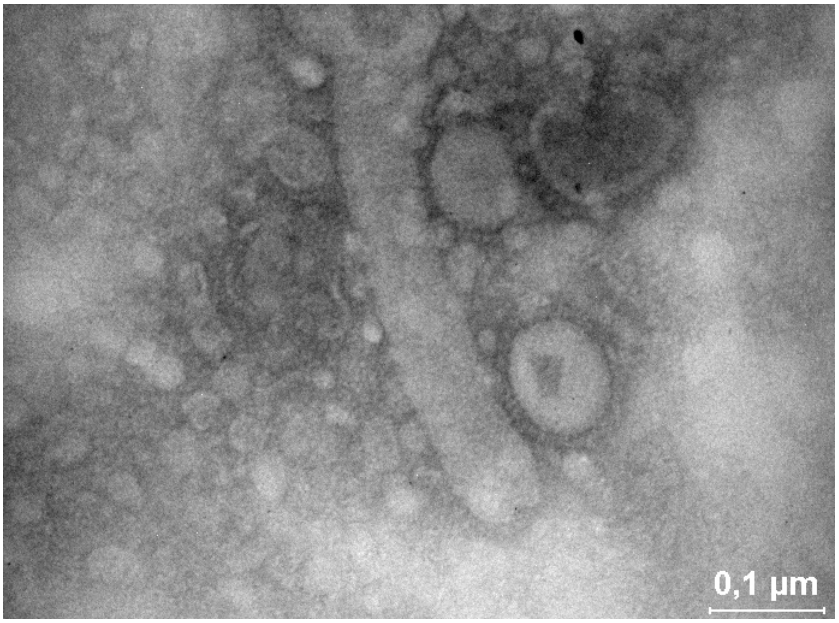


**Fig. 1.** Ultrastructure of the no frozen AIV virus samples (filamentous form, circular virions) on the fifth passage cultivation in chicken embryos.

Lethal virus activity after freezing of the samples stored not longer than 25 days was 8.31 ± 0.02 $ELD_{50}$/0.2 ml. Hemagglutination titers within these storage periods at –20°C did not differ from the baseline. Lethal virus activity after freezing –70 ± 2°C of the samples stored 143 months was 8.0 ± 0.01 and 7.5 ± 0.01 $ELD_{50}$/0.2 ml.

In all the cases of storage temperature and duration we identified such a feature of AIV infecting the chicken embryos as hemorrhages in serous and mucous membranes and organs of chicken embryos, regarded as a pathognomonic sign of AIV (Fig. 2).

Hemagglutination virus activity after freezing –20 ± 0.5, –70 ± 0.5, –196 ± 0.1°C of the samples stored for 25 and 115 days was (1:512), it did not differ from the baseline. After 18 months at –20 ± 0.5°C viral titer in hemagglutination was (1:256). After 210 days at –20 ± 0.5°C viral titer in hemagglutination was zero.

Ultrastructure of AIV virus samples stored for 18 months at –20°C presented viruses with damaged spicules of hemagglutinin on the surface of virions (Fig. 3).

Hemagglutination virus activity after freezing –70 ± 2°C of the samples stored 143 months was (1:128), it differed from the baseline (1:1024).

Infectious virus activity after thawing of the samples stored for 143 months at –70 ± 2°C was slightly reduced and made 8.0 ± .03 $EID_{50}$/ml.

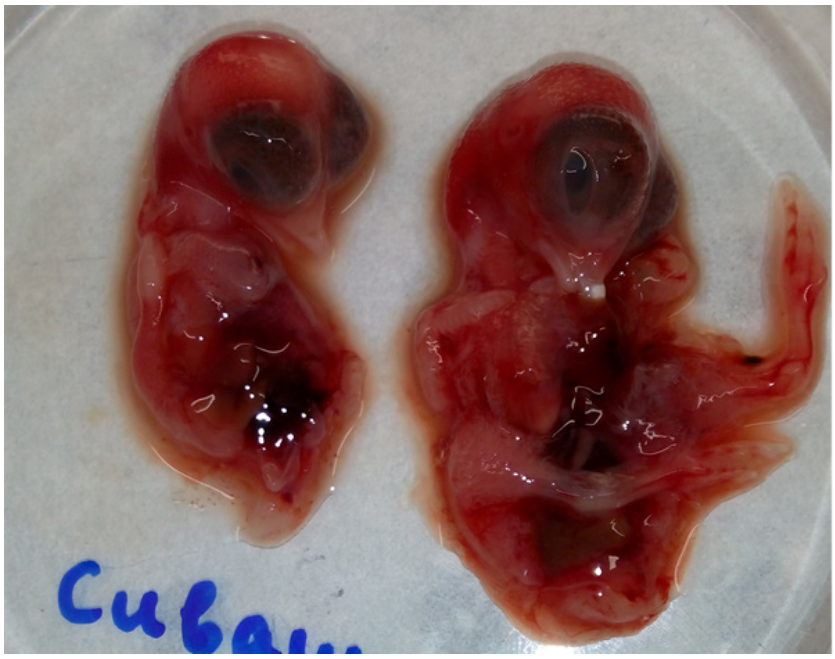

**Fig. 2.** Hemorrhages in serous and mucous membranes and organs of AIV infected chicken embryos.

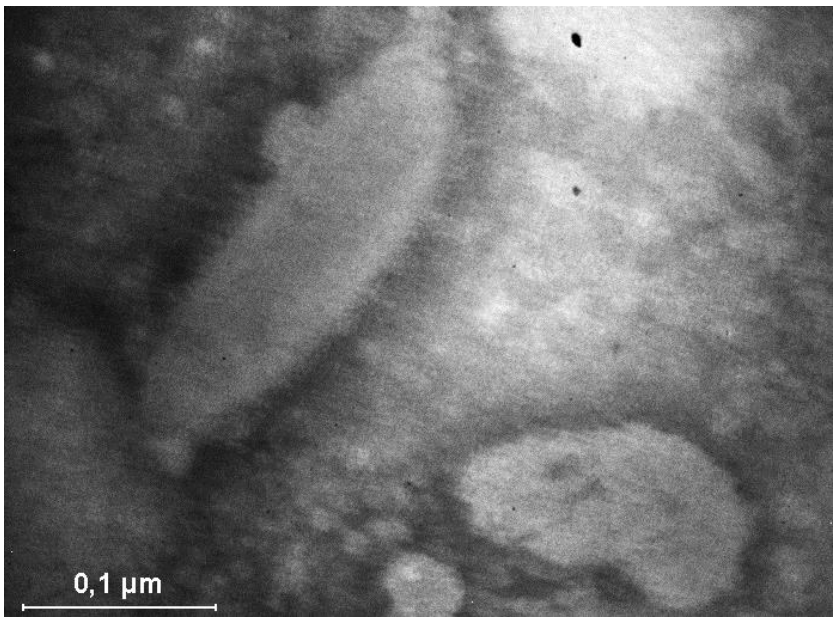


**Fig. 3.** Ultrastructure of AIV virus samples stored for 18 months at –20°C (viruses with the partly damaged hemagglutinin on the surface of virions).

The analysis of electronograms of the viruses, stored for 18 months at –20°C, showed the presence of polymorphic virions with a distinct bilayer lipoprotein envelope, but the glycoprotein processes, forming the spicules of hemagglutinin were absent (Fig. 3).

During freezing the virions possessing a total electric charge of a certain value (Oparin, 1996) occur to the water-ice phase transition zone, where they are exposed to an external electric field effect. It can be assumed that during long-term storage of AIV at –20°C the structure of virions could be destabilized because of the conformational changes associated not only with an alteration in their shape, but also with intramolecular mobility of structural components of the virion protein molecules.

The researches of Lugovoy (1985) on the mechanisms of freezing damaging effect on proteins, in particular enzymes, have shown that one of the main causes of the disordered structural and functional properties of enzyme proteins are high concentrations of inorganic salts, appearing during water crystallization in the solution, which possess the lyotropic properties as well as provide the pH shifts during the water freezing-out. The author believed that the structural basis of cryostability or cryolability in enzyme proteins was stipulated by an unequal degree of conformational mobility and rigidity of macromolecules, which, in turn, was caused (in accordance with the principle of thermo-dynamic free energy minimum) by different features of the amino acid composition of the protein molecule polypeptide chain.

The absence of peplomers on the electronograms of AIV stored for 18 months at –20°C, explains the total loss of hemagglutinating properties.

The ultrastructure of AIV, long-term-stored at low temperatures (–70 and –196°C) was more intact than after storage at –20°C and was close to the ultrastructure of the virus which was shortly stored (115 days) at a temperature of refrigerator.

All this testifies to the fact that the lower the storage temperature of cryopreserved viruses is, the more fully their ultrastructure and biological properties are retained. Electron microscopy studies allowed a visual assessment of the contribution of disordered structural integrity of the virions in the cryoinjuries of viruses during their cryopreservation and low temperature storage as exemplified by AIV.

**Conclusions.** The conducted study allowed to reveal on electronograms the ultrastructural changes in AIV during long term storage (18 months) at moderately low temperature (–20°C), in particular loss of glycoprotein of peplomers in the majority of virions.

The changes in ultrastructure of the virus samples were accompanied by a loss of hemagglutinating activity during long-term storage of AIV samples at moderately low temperature of –20°C.

When storing the AIV samples at –70 and –196°C the virions generally remain negatively contrasted, keep peplomers for the studied storage duration.

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# Part 3. Biosafety

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## EMBRYOTOXIC AND TERATOGENIC EFFECTS OF 'VITOSEPT' ON WHITE RATS

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**Summary.** The effect of 'Vitosept' drug, based on sodium hypochlorite solution with high purity, obtained in a specially developed membraneless flow electrolyzer, on the embryotoxic and teratogenic properties in rats was investigated. To determine the embryotoxic effect of 'Vitosept' on the development of white rats offspring of the 1st generation, control and three experimental groups ($G_1$, $G_2$, $G_3$) were formed from pregnant females. The females of the control group with a blunt probe were injected daily for 30 days with 5 ml of isotonic sodium chloride solution, and experimental ones with 5 ml of 'Vitosept' drug with different concentration of high purity sodium hypochlorite: Group I ($G_1$) — 50 mg/l; Group II ($G_2$) — 100 mg/l; Group III ($G_3$) — 500 mg/l. The animals were observed. During the observation the condition and behavior of the females, the dynamics of body weight change, duration of pregnancy, and the course of birth were monitored. The results of the experiment were recorded after the slaughter of pregnant females (20th day of pregnancy) and in the postnatal period of development of the offspring. Studies have shown that the use of different concentrations of the drug 'Vitosept' in rats for 30 days before and during pregnancy has no embryotoxic and teratogenic effects. According to the indicators of the total, pre- and postimplantation lethality of embryos, there were no reliable changes in the structure and morphometry of internal organs and tissues in 20-day-old fetuses, and their development corresponded to the terms of pregnancy. There was no significant difference between the fertility of female rats in the test and control groups. The average number of fetuses per female was within 9 animals. The rats obtained from the females of the experimental groups were viable and did not lag behind in growth and development compared with the control animals, which generally characterizes the studied drug 'Vitosept' as non-toxic, lacking embryotoxic and teratogenic action

**Keywords:** sodium hypochlorite, white rats, embryotoxic effect, teratogenic action

**Introduction.** Over the last 25–30 years, sodium hypochlorite solutions with high purity have become increasingly active in medicine and veterinary medicine. We have gained a lot of positive experience in their use, expanding the scope, developing new treatments (Kotsiumbas and Velichenko, 2009).

In-depth pharmaco-toxicological and morpho-functional studies by the staff of the State Scientific Research Institute of Veterinary Drugs and Feed Additives (Kotsiumbas et al., 2006; Kotsiumbas and Kotsiumbas, 2000) have made it possible to compare the properties of solutions of high-purity hypochlorite obtained at different facilities, to reveal new important features of their use for the treatment of animals.

For the study, we used 'Vitosept', based on a sodium hypochlorite solution with high purity, obtained in a specially developed membraneless flow electrolyzer in the process of direct electrochemical reaction, bypassing the formation of molecular chlorine. An isotonic sodium chloride solution (0.9% NaCl) prepared on water purified by special technology was used as the starting electrolyte. Such solutions do not contain impurities of organic matter and transition metal ions. The resulting sodium hypochlorite solution of high-purity is the optimal carrier of active oxygen.

Preclinical study of the drug harmlessness involves the study of embryotoxic risks, which is one of the integral safety criteria in the use of the drug at any stage of life of animals since the complexity of the phenomenon of reproduction makes the organism very susceptible. Such researches make it possible to establish the nature and severity of the harmful effect of drugs on the organism of the fetuses of experimental animals, depending on the period of intrauterine development, and, at the same time, to evaluate the safety of its use during pregnancy (Baryliak et al., 2001; Dyban, 1986; Durnev and Seredenin, 1998).

**The purpose of the study** was to identify possible embryotoxic and teratogenic effect of the drug 'Vitosept' with long-term intragastric input to white rats.

**Materials and methods.** The experiments on laboratory rats were conducted in the vivarium of the State Research Institute of Veterinary Drugs and Feed Additives (Lviv, Ukraine). To determine the embryotoxic effect of 'Vitosept' on the development the of white rats offspring of the 1st generation, a control (C) and three experimental groups ($G_1$, $G_2$, $G_3$) were formed from pregnant females.

The females of the control group were injected with a blunt probe daily for 30 days with 5 ml of isotonic sodium chloride solution, and experimental ones with 5 ml of 'Vitosept' drug with different concentrations of hypochlorite sodium of high purity: Group I ($G_1$) — 50 mg/l; Group II ($G_2$) — 100 mg/l; Group III ($G_3$) — 500 mg/l.

Experiments on animals were carried out in accordance with with the rules of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986).

The animals were observed. During the observation the condition and behavior of the females, the dynamics of body weight change, duration of pregnancy, and the course of birth were monitored. The results of the experiment were recorded both after slaughter of pregnant females (20$^{th}$ day of pregnancy) and in the postnatal period of the offspring development (Benz and Beltz, 1980; Hayashi, Sofuni and Ishidate, 1984).

After euthanasia, under the light ether anesthesia of the animals, an incision of the abdominal cavity and the uterine horns was made by dislocation of the cervical vertebrae. The following indicators were taken into account: the number of yellow bodies of pregnancy, the number of living fetuses, the number of dead fetuses, the number of sites of resorption, the state of the placenta. The fetuses and placenta were weighed, the coccygeal parietal (cranio-caudal) distance was measured, the mass growth coefficient of the fetus and the placental coefficient were calculated. The results of the autopsy of the pregnant females were recorded in the protocol. After laparotomy, the horns of the uterus with the ovaries were excised in the animals. They were transferred to a Petri dish with saline solution. With the help of binocular magnifier, a thorough examination of the ovaries was performed, the number of yellow bodies of pregnancy was calculated.

Based on the results of the autopsy according to the Malashenko and Egorova formulas we determined the following indicators:

a) total embryonic mortality (%) — (C–A):C×100;

b) preimplantation mortality (%) — [C–(A+B):C]×100;

c) postimplantation mortality (%) — B:(A+B)×100,

where: A — is the number of living fetuses; B — the number of dead and resorbed embryos; C — the number of yellow bodies of pregnancy.

Indicators of the embryotoxic effect of the 'Vitosept':

a) embryonic (pre- and postimplantation) death of fetuses;

b) developmental delay, which is manifested in the reduction of body weight of cranio-caudal fetus sizes;

c) appearance of pathology of internal organs development;

d) appearance of external anomalies (teratogenic effect).

The teratogenic effect of the studied drug was examined initially visually (in the process of autopsy of pregnant females) (Il'inskikh et al., 1990). Subsequently, part of the fetus (⅔ of the total number of fetuses in the experiment) was fixed in 95% ethyl alcohol for further evaluation of the condition of the bone system on enlightened substances, painted with alizarin by the Dawson method. The remaining fetus were immersed in a Bowen fluid for microanatomical analysis by Wilson method. When newborn rats were examined, cranio-caudal size was recorded, body weight was determined. The cases of death of rats from the moment of birth to the termination of feeding were taken into account. We studied the postnatal development of offspring from rats of experimental and control groups by the following indicators: dynamics of increase in body weight, appearance of eyes, viability index (number of live births/number of births), lactation index (number of live rats up to the 4$^{th}$ and 21$^{st}$ days), survival index (number of rats surviving up to the 4$^{th}$ day/number of rats born alive). On the 30$^{th}$ day after the birth, rats were euthanized by dislocation of the cervical vertebrae, and histologic examination of the internal organs was conducted. Statistical processing of the results was performed by mathematical statistics using the application packages 'Biostatistics for Windows' (v. 4.03) and 'Microsoft Excel 2002'. The arithmetic mean (M) and standard deviation of the arithmetic mean (m) were determined for each indicator.

**Results.** Experiments show that during 20 days of pregnancy, the deaths of female rats in the experimental groups were not recorded, they were active and ate well.

As can be seen from the data in Table 1, the dynamics of weight gain of females in all groups indicates its increase during the entire pregnancy period. At the same time, for 20 days the most increase in body weight was in the animals of the experimental groups $G_1$, $G_2$, and $G_3$, by 30.8, 31.4, and 30.0%, respectively, compared with their body weight at the beginning of the experiment.

**Table 1 —** Dynamics of weight gain of pregnant females under conditions of application of different concentrations of the drug 'Vitosept' (M ± m, n = 5)

| Groups | Periods of experience, days | | | |
|---|---|---|---|---|
| | 0 | 10 | 15 | 20 |
| C | 198.8 ± 1.44 | 199.6 ± 5.37 | 217.0 ± 10.2 | 254.6 ± 15.09 |
| $G_1$ | 198.4 ± 1.71 | 202.3 ± 8.19 | 228.6 ± 13.8 | 259.5 ± 23.9 |
| $G_2$ | 195.3 ± 2.19 | 198.5 ± 2.23 | 218.4 ± 3.39 | 256.7 ± 14.3 |
| $G_3$ | 187.8 ± 2.41 | 195.5 ± 7.17 | 207.9 ± 8.23 | 245.8 ± 8.94 |

During the macroscopic examination of the fetus, both the animals of the control and the experimental groups showed no lag of their development, in relation to the term of pregnancy, on the 20th day. It was found that the fetus membranes were properly formed, the amniotic fluid was transparent, the placenta was full-blooded. When the fetal shells were dissected and the umbilical cord was cut, the fetuses began to breathe independently. The skin was pink in color and slightly wrinkled in appearance. All embryos of the control and experimental groups had no noticeable defects in the structure of the skull and trunk. The back was straight. The skull had an oval-oblong shape. The auricle and the eyelids were closed. The anterior abdominal wall was fused, with no signs of umbilical hernia. The tail was of ordinary length. The limbs had a well-developed shoulder, forearm, brush, thigh, lower leg, and foot. The position, the shape of the extremities, the number of fingers in the test and control embryos were within the normal range.

The results of the study of embryonic material obtained from pregnant females under different concentrations of 'Vitosept' are shown in Table 2.

**Table 2 —** The results of the study of embryonic material from pregnant females under different concentrations of 'Vitosept' (M ± m, n = 5)

| Indicators | Groups | | | |
|---|---|---|---|---|
| | C | $G_1$ | $G_2$ | $G_3$ |
| The amount of live fetus in the placenta | 9.8 ± 0.51 | 9.7 ± 0.37 | 9.1 ± 0.28 | 9.5 ± 0.54 |
| Total embryonic mortality, % | 8.13 ± 0.73 | 7.29 ± 0.81 | 7.42 ± 0.32 | 8.35 ± 0.95 |
| Preimplantation mortality, % | 10.1 ± 0.65 | 9.32 ± 0.43 | 9.34 ± 0.68 | 8.93 ± 0.42 |
| Postimplantation mortality ,% | 8.14 ± 0.51 | 9.1 ± 0.37 | 9.3 ± 0.28 | 9.5 ± 0.44 |
| Weight of the fetus, g | 2.53 ± 0.23 | 2.29 ± 0.31 | 2.12 ± 0.32 | 2.15 ± 0.75 |
| Craniocaudal dimensions, mm | 30.7 ± 0.65 | 30.2 ± 0.43 | 30.4 ± 0.68 | 30.1 ± 0.42 |
| Placental mass, g | 0.41 ± 0.51 | 0.41 ± 0.37 | 0.42 ± 0.28 | 0.42 ± 0.54 |
| The diameter of the placenta, g | 13.3 ± 0.73 | 13.2 ± 0.81 | 13.4 ± 0.32 | 13.3 ± 0.25 |
| External defects of development, % | 0 | 0 | 0 | 0 |

We have found that the embryogenesis indices of rats in the experimental group were similar to those of control animals. In particular, the weight of the fetuses on the 20th day of pregnancy fluctuated in the control group from 2.5 g, and in the experimental groups from 2.12 to 2.29 g. The mean values of this indicator in all groups did not differ statistically.

During the study of the development of internal organs by the method of Wilson, it was found that all the fetuses, born by females of the control and experimental groups had no pathological abnormalities, and their development corresponded to the term of pregnancy.

In the first incision, which was performed perpendicular to the mandible, the condition of the anterior compartment of the rigid palate, mandible, and nasal septum were studied. In all embryos examined, the lower and upper jaws were free of pathologies, the tongue was freely placed in the mouth. The rigid palate had no signs of splitting, the nasal septum was not curved.

The second incision was made through the middle of the eyeballs and orbits. It is found that the olfactory vesicles are located in the frontal part of the brain — large, with incisions, have an oblong-oval shape. Ocular orbits and apples are paired at the same level, without pathology.

The third incision was made through the transverse diameter of the brain (in front of the ears), the fourth incision was parallel to the third, but behind the ears. These sections examined the condition of the brain. It is noted that in all embryos the brain sections are developed proportionally. The incisions revealed hemispheres, thalamus (midbrain), cerebellum, lateral, third and fourth ventricles of the brain. The lateral ventricles of the brain looked like a narrow slit. The third ventricle on the incision was small, droplet-shaped. The fourth ventricle had a flattened, tent-like shape.

The results of morpho-anatomical study of embryos under the conditions of application of different concentrations of the drug 'Vitosept' are given in Table 3.

**Table 3 —** Effect of 'Vitosept' on the morphofunctional state of rat embryos

| Indicators | Groups | | | |
|---|---|---|---|---|
| | C | $G_1$ | $G_2$ | $G_3$ |
| Number of examined fetus | 98 | 97 | 95 | 91 |
| Blood vessels overflow, units/% | 0/0 | 0/0 | 1/1.02 | 1/1.05 |
| Increased bladder, units/% | 1/0.65 | 0/0 | 1/1.03 | 2/1.42 |
| Internal hemorrhage, unit/% | 1/1.04 | 0/0 | 1/1.03 | 0/0 |
| Extension of the ventricles of the brain, units/% | 1/1.04 | 0/0 | 0/0 | 0/0 |
| Subcutaneous hematomas, units/% | 1/1.04 | 0/0 | 1/1.03 | 0/0 |
| Injuries, skeletal development anomalies, units/% | absent | absent | absent | absent |

The subdural space in all the fetuses did not exceed the limit of normal. In two fetuses of the control group and

one fetus from $G_3$, blood filling of the blood vessels of the brain was observed, and in one embryo of $G_1$ enlargement of the ventricles of the brain was recorded. Subcutaneous hematomas were detected in one of the embryos of the $G_2$ and control groups. The edema of the subcutaneous tissue in the fetus of the experimental and control groups was absent.

The fifth incision was made through the larynx, esophagus, spinal cord, large blood vessels, and salivary glands. All of the above objects had normal topography with no apparent pathology. The subarachnoid space was normal, the diameter of the blood vessels was approximately the same in all embryos of the experimental and control groups.

The sixth incision was made over the upper extremities. It was followed by the study of the condition of the esophagus, trachea, blood vessels, spinal cord. At this level of incision, no visible pathology was detected. The esophagus throughout was free, without signs of stenosis, the tracheal rings were well developed, with normal topography.

The seventh incision was made under the upper extremities. The incision clearly showed the organs of the thoracic cavity: four-chambered heart, right and left ventricles, right and left ear, right lung (consisting of four lobes) and monoclonal left lung. The lung tissue itself had a well-defined cellular structure, the bronchi were developed. In the pericardial cavity, in some embryos, both in the experimental and control groups, the presence of blood was noted. The same section also examined the condition of the esophagus and spinal cord. All organs were of normal topography and size.

The eighth incision was made through a six-particle liver that had the usual consistency and color. After examination, the liver was removed and the diaphragm was examined. The diaphragm partition had a slightly concave shape, its integrity was not broken. There was found that one of the embryos in $G_1$ and one in the control group had hemorrhage into the internal organs.

The ninth incision in part of the embryos was performed below the umbilical ring, and in the other, along the abdominal cavity and pelvis. In both transverse and longitudinal sections, the abdominal organs had corresponding topography, with no signs of pathology. Stomach — large, slightly folded. The pancreas is compact, with a well-visible (longitudinal section) head, body and tail. The spleen was normal, moderate in size. After removing these organs, we examined the genitourinary system. The kidneys in all embryos were located somewhat asymmetrically. In the incision, the renal pelvis had no evidence of hydronephrosis. The large adrenal glands were oval in shape. The ureter is straight throughout. The bladder was small in size, however, in several fetuses in the control and $G_2$ and $G_3$ groups, the size of the bladder was increased. The rectum had no signs of pathology. In males clearly developed paired testicles with appendages were observed, in females — uterus and ovaries (located behind the kidneys).

The results of the morpho-anatomical study of the internal organs of 20-day-old embryos of rats, which received different concentrations of 'Vitosept' during pregnancy, showed the absence of reliable changes in internal organs and tissues compared to control.

When examining the bone system of embryos, the character of ossification of the skull, skeleton, extremities, the spatial location and shape of the bones, the number of anlages in the metacarpal and metatarsal bones, the number of ossifications in the sternum, pelvic girdle and spine were determined.

Bone tissue was found to have a bright color. In the individual embryos of the studied groups, the absence of the upper part of the occipital bone was detected, but the detected deviations did not go beyond the analogous control indices. In the embryos of both the experimental and control groups, a decrease in bone anlages in the sublingual bone were observed. In the course of the embryo spine study, no significant ossification disorders were detected in the fetuses of the experimental groups, compared with the embryos of the control group. The number of pairs of ribs is 13. Only the arches of the vertebrae are ossified in the cervical section. In the thoracic, lumbar, and sacral sections, ossification anlages were found in both the arches and vertebral bodies.

After birth, at 1st, 5th, 10th, and 20th days of age, the dynamics of body weight was determined, and morphometry was performed. The results of the studies are shown in Table 4.

**Table 4 —** Body weight (mg) of white rats of 1st generation from females, which received different concentrations of the drug 'Vitosept' (M ± m, n = 5)

| Days of experience | Groups | | | |
|---|---|---|---|---|
| | C | $G_1$ | $G_2$ | $G_3$ |
| 1 | 4.94 ± 0.12 | 4.89 ± 0.18 | 4.93 ± 0.07 | 4.89 ± 0.27 |
| 5 | 8.6 ± 0.57 | 8.9 ± 0.04 | 8.2 ± 0.13 | 8.5 ± 0.76 |
| 15 | 27.2 ± 2.57 | 26.8 ± 0.58 | 27.2 ± 0.54 | 26.2 ± 1.99 |
| 20 | 45.3 ± 2.18 | 44.6 ± 2.25 | 44.9 ± 0.71 | 44.8 ± 1.31 |

As can be seen from the results of the studies, the bodyweight gain in rats of all groups was quite high. In particular, on the 20th day of the experiment the bodyweight of the animals of the experimental groups increased by 7.4, 6.8, and 6.6 times, respectively. However, no significant difference between the body weight of the animals of the experimental and control groups was noted.

The dynamics of postembryonic development rates are shown in Table 5.

**Table 5 —** Effect of 'Vitosept' on the morpho-anatomical postembryonic development of the fetus

| Indicators | Groups | | | |
|---|---|---|---|---|
| | C | $G_1$ | $G_2$ | $G_3$ |
| Average number of fetus per female | 9.49 ± 0.27 | 9.19 ± 0.17 | 9.20 ± 0.33 | 9.33 ± 0.25 |
| Weight of baby-rats on the first day, g | 4.94 ± 0.12 | 4.89 ± 0.18 | 4.93 ± 0.07 | 4.89 ± 0.27 |
| The average daily increase in baby-rats, g | 1.54 ± 0.07 | 1.49 ± 0.8 | 1.49 ± 0.07 | 1.50 ± 0.07 |
| The appearance of wool, day | 5 | 5 | 5 | 5 |
| Ear shell detachment, day | 13 | 13 | 13 | 13 |
| Opening of the palpebral fissure, day | 16 | 16 | 16 | 16 |
| Viability Index | 1 | 1 | 1 | 1 |
| Survival index | 1 | 1 | 1 | 1 |
| Lactation index | 1 | 1 | 1 | 1 |

It was found that the wool appeared in control and experimental animals at the same time, 5 days after birth. Eye openings in control and experimental rats were recorded at the age of 16 days. The eruption of the auricle was recorded on the 13$^{th}$ day of life.

**Conclusions**. Studies have shown that the use of different concentrations of the drug 'Vitosept' in rats for 30 days before and during pregnancy has no embryotoxic and teratogenic effects.

According to the indicators of the total, pre- and post-implantation lethality of embryos, there were no reliable changes in the structure and morphometry of internal organs and tissues in 20-day-old fetuses, and their development corresponded to the terms of pregnancy. There was no significant difference between the fertility of female rats in the test and control groups. The average number of fetuses per female was within 9 animals.

The rats obtained from the females of the experimental groups were viable and did not lag behind in growth and development compared with the control animals, which generally characterizes the studied drug 'Vitosept' as non-toxic, lacking embryotoxic and teratogenic action.

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# Contents

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