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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 (G₁, G₂, G₃) 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 (G₁) — 50 mg/l; Group II (G₂) — 100 mg/l; Group III (G₃) — 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 of white rats offspring of the 1st generation, a control (C) and three experimental groups (G₁, G₂, G₃) 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 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 (20th 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:

- total embryonic mortality (%) — $(C-A):C \times 100$;
- preimplantation mortality (%) — $[C-(A+B):C] \times 100$;
- postimplantation mortality (%) — $B:(A+B) \times 100$,

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':

- embryonic (pre- and postimplantation) death of fetuses;
- developmental delay, which is manifested in the reduction of body weight of cranio-caudal fetus sizes;
- appearance of pathology of internal organs development;
- 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 ($\frac{2}{3}$ 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 4th and 21st days), survival index (number of rats surviving up to the 4th day/number of rats born alive). On the 30th 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' ($M \pm m$, $n = 5$)

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' ($M \pm m$, $n = 5$)

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 G₃, blood filling of the blood vessels of the brain was observed, and in one embryo of G₁ enlargement of the ventricles of the brain was recorded. Subcutaneous hematomas were detected in one of the embryos of the G₂ 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 G₁ 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 G₂ and G₃ 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 anlagen 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 anlagen 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 anlagen 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 13th 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.

References

- Baryliak, I. P., Neumerzhyska, L. V., Byshovets, T. F. and Danylenko, B. C. (2001) ‘Study of the gonadotoxic effects of new drugs and their influence on the animal reproductive function’ [Vyvchennia honadotoksychnoi dii novykh likarskykh zasobiv ta yikh vplyvu na reprodutyvnu funktsiiu tvaryn], in Stefanov, O. V. (ed.) *Preclinical Studies of Drugs [Doklinichni doslidzhennia likarskykh zasobiv]*. Kyiv: Avitsenna, pp. 139–152. [in Ukrainian].
- Benz, R. D. and Beltz, P. A. (1980) ‘Cytogenetic toxicologic testing with dogs’, *Environmental Mutagenesis*, 2(2), pp. 312–313. doi: 10.1002/em.2860020214.
- CE (The Council of Europe). (1986) *European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes*. (European Treaty Series, No. 123). Strasbourg: The Council of Europe. Available at: <https://conventions.coe.int/treaty/en/treaties/html/123.htm>.
- CEC (The Council of the European Communities). (1986) ‘Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes’, *The Official Journal of the European Communities*, L 358, pp. 1–28. Available at: <http://data.europa.eu/eli/dir/1986/609/oj>.
- Durnev, A. D. and Seredenin, S. B. (1998) *Mutagens. Screening and Pharmacological Prevention of Effects [Mutageny. Skrining i farmakologicheskaya profilaktika vozdeystviy]*. Moscow: Meditsina. ISBN 5225044298. [in Russian].
- Dyban, A. P. (ed.) (1986) *Methodological Guidelines for the Study of the Embryotoxic Action of Pharmacological Substances and the Effect on Reproductive Function [Metodicheskie ukazaniya po izucheniyu embriotoksicheskogo deystviya farmakologicheskikh veshchestv i vliyaniya na reprodutyvnoy funktsiyu]*. Moscow. [in Russian].
- Hayashi, M., Sofuni, T. and Ishidate, M. (1984) ‘Mechanism of micronuclei formation in mouse bone marrow’, *Mutation Research*, 130(5), p. 364. doi: 10.1016/0165-1161(84)90031-1.
- Il’inskikh, N. N., Medvedev, M. A., Bessudnova, S. S. and Il’inskikh, I. N. (1990) *Mutagenesis at Different Functional States of an Organism [Mutagenez pri razlichnykh funktsional’nykh sostoyaniyakh organizma]*. Tomsk: Tomsk University Press. ISBN 575110972. [in Russian].
- Kotsiumbas, I. Ya. and Kotsiumbas, G. I. (2000) ‘Features of morphological research in the study of new drugs’ toxicity’ [Osoblyvosti morfolohichnykh doslidzhen pry vyvcheni toksychnosti novykh likarskykh zasobiv], *Bulletin of the Bila Tserkva State Agrarian University [Visnyk Bilotserkivskoho derzhavnoho ahrarnoho universytetu]*, 13(2), pp. 79–84. [in Ukrainian].
- Kotsiumbas, I. Ya. and Velichenko, O. B. (eds.) (2009) *Prospects for the Use of Hypochlorites in Veterinary Medicine [Perspektyvy zastosuvannia hipokhlorytiv u veterynarnii medytsyni]*. Lviv: Afisha. ISBN 9789663251226. [in Ukrainian].
- Kotsiumbas, I. Ya., Malyk, O. H., Patereha, I. P., Tishyn, O. L. and Kosenko, Yu. M. (2006) *Preclinical studies of veterinary drugs [Doklinichni doslidzhennia veterynarnykh likarskykh zasobiv]*. Lviv: Triada plus. ISBN 9667596648. [in Ukrainian].