# EXPERIMENTAL STUDY OF PREPARATION ENTEROSGEL ON PREGNANCY IN RATS AND THEIR OFFSPRING

**N.A. Cornuta** Ph.D, Biology, **N.V. Kokshareva**, Doctor of Science, Biology, **V.G. Nikolaev**, Habilitation degree in Medicine
/L.I. Medvedya Institute of ecohygiene and toxicology;
Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kiev /

## Introduction

Pregnancy is a specific state in woman's life that needs attention, particularly in relation to clinical and pharmacological aspects. Mother-placenta-fetus triad should be considered as a single biological, pharmacokinetic and pharmacodynamic complex. Therefore, taking care of mother's health requires taking into account the possible impact on mother and fetus while performing the preventive and therapeutic measures [1]. Mother's body is the main object when taking medications during pregnancy, and in fact, the fetus is an unwitting recipient. This dependence is maintained throughout the pregnancy. Therefore, it is important to care not only about the health of pregnant women but at the same time consider possible consequences of treatment to the developing fetus, as the embryo or fetus has a unique sensitivity to specific drugs. And this sensitivity is not comparable with that of an adult organism.

Among many compounds that show embryotoxic properties (chemical carcinogens, heavy metals, chlororganic and fluoroorganic compounds, hydrocarbons, sulfur compounds, amides, amines), prominent place is held by medical products: hormonal drugs, sleeping pills, sedating medication, tranquilizers, antibiotics and anticancer drugs [2]. It is common knowledge that any risk of possible embryo or fetus damage is an exorbitant price for the use of drugs during pregnancy [3]. Therefore, one of the key requirements for each new drug is the absence of any negative impact on the fetus development [4]. The use of Enterosgel preparation (hydrogel methylsilicic acid) is recommended for pregnant women in toxicosis of the first half of pregnancy. The literature data with regard to its influence on embryo development is very limited. There are few reports on the absence of fetotoxicity in Enterosgel preparation [6].

**Objective**: The study is to determine the toxicity of Enterosgel preparation on pregnant doe rats, as well as its embryotoxicity and teratogenic action..

## Materials and methods

The study was conducted on 100 white adult doe Wistar (weight 210-240 g) rats obtained from the PE "Biomodelservis" nursery and kept in quarantine for 2 weeks.

During the experiment animals were kept in vivarium in standard plastic cages in groups of 6 individuals, at a temperature of 20-22°C, 50-60% humidity, standard light conditions "day-night" at ambient temperature. Animals received standard briquetted feed in compliance with the established feeding regime and ad libitum water. The sperm detection in vaginal smears was considered as the first day of pregnancy.

The study of embryotoxic and teratogenic properties of Enterosgel preparation was conducted in accordance with the methodology recommendations on preclinical studies of medical products [7]. The intragastric administration of Enterosgel was performed on experimental animals. The assessment of toxicity indices for pregnant doe rats with Enterosgel preparation was carried out in comparison with

the control group of rats that received purified water in a similar dose. Two doses were taken for the study: the maximum daily therapeutic dose for the human - 45 g / kg and 5-fold exceeding dose - 225 g / kg (in terms of laboratory animals these doses are 643 mg / kg bw and 3.21 g / kg bw, respectively). Dose was adjusted according to the body weight change of pregnant doe. The preparation was administered to animals in the morning on an empty stomach without solvent use: 1st to 6th, 6th to 16th, 16th to 20th day of pregnancy (1, 2 and 3 groups respectively). Animals of the first and third groups were administered the preparation in a single dose (maximum - 3.21 g / kg bw), the second group - in both doses (643 mg / kg bw and 3.21 g / kg bw - group 2-A and 2-B).

Health observation of pregnant doe was carried out before the injection (including zero, 3rd and 5th days of the expected gestation), several times a day during the period of drug administration (continuously within the first hour after drug administration; 6 hours after drug administration) and daily during morning hours after treatment cessation throughout the experiment. The measurement of animal body weight in experimental and control groups was performed in the dynamics of the 1st, 6th, 13th and 21st days of pregnancy. Furthermore, at the end of the experiment the effect of the drug was evaluated on the absolute and relative mass of internal organs, on the enzymatic system of the liver (by the activity of alanine aminotransferase and aspartate transaminase, alkaline phosphatase), the protein content level and thymol test in the blood serum of experimental rats was completed and compared with control group of animals.

Euthanasia of animals was carried out on the 21st day of gestation. The number and placement of corpus luteum in the ovaries were registered during the autopsy of pregnant doe. The number and location of implantation sites, early and late resorptions, and the number of live and dead fetuses were examined in the uterus. Embryonic mortality was calculated: codes of pre- and post-implantation mortality by standard formulas. The viability assessment was performed, fetus and its placenta were weighed and then the examination of fetuses was carried out to identify external malformations. One group of fetuses (2/3) was fixed in 96% alcohol and after the clarification by alkali solution, water flushing, it was stained with alizarin until pale violet color to study bony skeleton by Dawson method [7]. The accuracy of initiation and ossification of all fetal skeleton areas was assessed consistently. Another group (1/3) was fixed in Bouin's fluid and used for the study of internal organs on microanatomical sections by Wilson method [8, 9].

Generalized tables were based on 100 individual cards containing information on the analysis of 1160 fetuses. Study results were processed by the Student's t-test for normal distribution characteristic and by the Mann-Whitney test in case if the distribution law deviated from the normal. The database was formed in Microsoft Excel program. Calculations were made in Microsoft Excel and Biostat [10].

**Results and discussion**

The study of pregnant rats condition did not reveal the presence of general toxical action of Enterosgel preparation in their body when administering investigated doses at various pregnancy stages. The preparation had no effect on the consumption of water and food by animals. Intragastric administration of the preparation exercised no influence on the dynamics of body weight and body weight gain (Figure), the absolute and relative weight of organs of pregnant doe rats (Table 1).

As shown in Figure 1, during the experiment no statistically significant differences were detected in body weight and body weight gain of pregnant doe rats of the experimental groups compared with control group of animals.

Data analysis presented in Table 1 shows that after repeated Enterosgel administration there were no statistically significant changes in the relative mass of internal organs of the experimental rats compared with control group ($p > 0.05$).

Conducted biochemical studies included the analysis of the parameters characterizing the functional state of the liver in experimental animals. Thus, the effect of Enterosgel on liver function was assessed by enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST),

alkaline phosphatase, the content of total protein and cholesterol in blood serum. Administration of Enterosgel preparation in tested doses to experimental animals did not cause statistically significant changes in ALT activity. However, when Enterosgel was administered in doses of 643 mg / kg bw (From 6th to 16th days of pregnancy) and 3.21 g / kg bw (From the 16th to 20th days of pregnancy) a statistically significant decrease in the activity of AST was observed (respectively 0.66 and 0.67 mmol / L / hour; Control - 0.86 mmol / l / h).

However, it is known that the reduction of AST has no clear diagnostic value, while an important indicator of hepatocyte injury is an increase of ALT and AST activity in serum [11]. Determination of alkaline phosphatase activity in blood serum reveals liver and bone abnormality. The main sources of enzyme in plasma are osteoblasts, hepatocytes and intestinal epithelial cells [12]. In the course of performed studies it was shown that administration of Enterosgel preparation in tested doses in various stages of pregnancy had no effect on the activity of alkaline phosphatase serum. Values of alkaline phosphatase activity in blood serum of pregnant doe rats in experimental groups did not demonstrate statistically significant differences compared to control animals.

Determination of total protein in serum is an indicator that reflects the role of the liver in protein metabolism. The functional state of the liver and kidneys, a number of diseases, metabolic disorders may affect at the concentration of plasma protein. The protein content in the blood serum of pregnant doe rats treated with Enterosgel preparation in a therapeutic dose (643 mg / kg bw) and 5-foldhigher dose (3.21 g / kg bw) in various stages of pregnancy, did not differ significantly from the control group of animals. The concentration of cholesterol in the serum depends on its metabolism, which may be affected by a number of factors, in particular - the state of the endocrine glands, liver and kidneys. Its exchange is closely related to lipid metabolism. As studies have shown, no significant changes in the concentration of cholesterol in the blood serum were observed upon the administration of Enterosgel preparation in doses of 643 mg / kg bw and 3.21 g / kg bw. .

**Table. 1.** Absolute and relative weight of organs of pregnant doe injected with the drug Enterosgel

| Indices | Control | Enerosgel doses | | | |
|---|---|---|---|---|---|
| | | 3.21 g/kg (1-6) | 643 mg/kg (61-16) | 3.21 g/kg (61-16) | 3.21 g/kg (161-20) |
| Number of pregnant doe | 20 | 20 | 20 | 20 | 20 |
| The final body weight | 329.7 | 330.6 | 329.5 | 329.5 | 335.6 |
| Liver, g | 11.4 | 10.9 | 10.7 | 10.9 | 11.3 |
| The relative liver weight (g/100 g) | 3.46 | 3.30 | 3.25 | 3.31 | 3.37 |
| Kidney | 1.52 | 1.43 | 1.46 | 1.43 | 1.46 |
| Relative kidney weight (g/100 g) | 0.46 | 0.43 | 0.44 | 0.43 | 0.44 |
| The adrenal glands (g) | 0.06 | 0.06 | 0.06 | 0.07 | 0.06 |

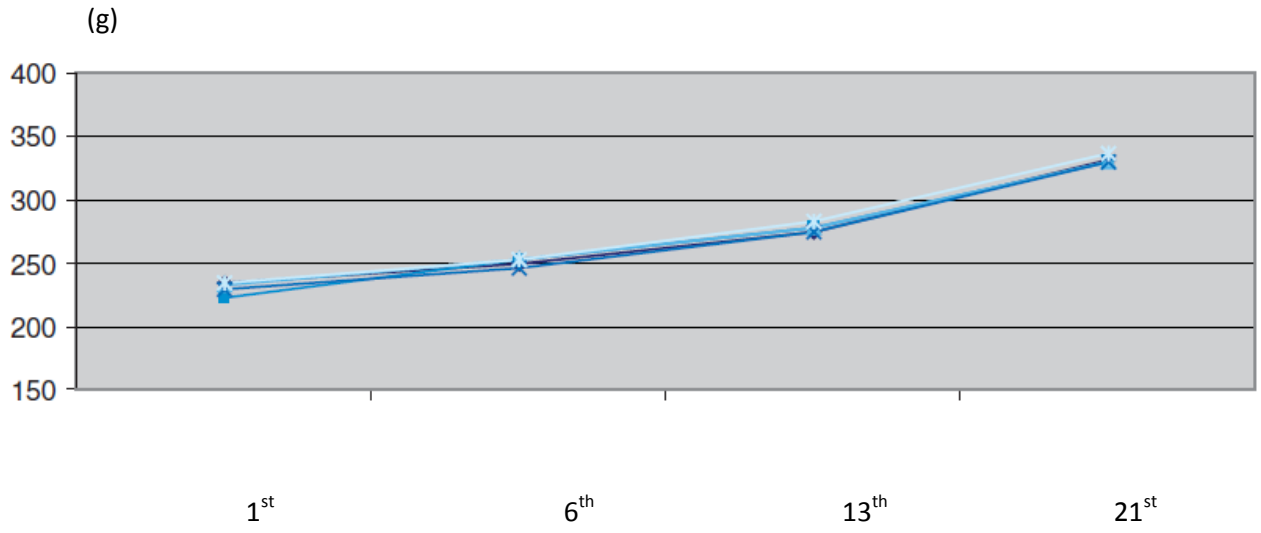


**Figure**. The body weight dynamics of pregnant doe rats (g) for intragastric administration of Enterosgel preparation

There were no reports on pregnant doe rats with complete resorption of embryos. The number of corpora lutea in the ovaries of experimental animals did not differ from control group. The preparation did not cause embryonic death, as evidenced by the absence of differences in the index of pre- and postimplantation death. The average number of viable fetuses per litter in the experimental and control groups did not differ (Table 2).

**Table 2.** Indicators of embryotoxic activity of Enterosgel preparation in experimental groups

| Indices | Control | Enterosgel doses | | | |
|---|---|---|---|---|---|
| | | 3.21 g/kg (1-6) | 643 mg/kg (6-16) | 3.21 g/kg (6-16) | 3.21 g/kg (16-20) |
| Total embryonic mortality, % | 15.1 | 11.9 | 6.5 | 12.6 | 11.4 |
| Death before implantation | 12.9 | 10.4 | 3.3 | 7.9 | 8.3 |
| Death after implantation | 2.5 | 1.2 | 3.0 | 4.7 | 3.3 |
| Index of perinatal development | | 1.02 | 1.08 | 1.02 | 1.05 |

The drug Enterosgel when administered at different stages of pregnancy had no effect on placental weight, body mass fetus and fetal-placental index (Table. 3).

**Table 3.** The results of morphometric studies of fetuses after Enterosgel administration

| Indices | Control | Enterosgel doses | | | |
|---|---|---|---|---|---|
| | | 3.21 g/kg (1-6) | 643 mg/kg (6-16) | 3.21 g/kg (6-16) | 3.21 g/kg (16-21) |
| The weight of the fetus | 3.80±0.20 | 3.87±0.20 | 3.88±0.20 | 3.90±0.20 | 3.91±0.20 |
| - tom | 3.82±0.20 | 3.88±0.20 | 3.88±0.30 | 3.91±0.20 | 3.93±0.20 |

| | | | | | |
|---|---|---|---|---|---|
| - doe | 3.80±0.20 | 3.85±0.20 | 3.86±0.20 | 3.90±0.30 | 3.87±0.20 |
| Placental weight | 0.57±0.07 | 0.57±0.03 | 0.57±0.03 | 0.57±0.04 | 0.57±0.02 |
| Fetal-placental index | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |

The data presented shows that Enterosgel did not exercise any negative influence on growth and development of the offspring rats.

At course introduction Enterosgel did not cause specific congenital anomalies of internal organs: brain, heart, lungs, liver, spleen, kidneys. At external examination no external anomalies of facial region and extremities were mentioned.

The variation frequency of internal organs development, such as the expansion of renal pelvis and ureter in fetuses of control and experimental groups were not significantly different, and corresponded to the indices described by other authors [13, 14]. The study of bone systems by Dawson method in all experimental groups revealed normal ossification and the absence of anomalies in development of vertebra, ribs, sternum, pelvis bones, bones of the limbs and other parts of the skeleton (Table 4).

**Table 4.** Summarized data on the analysis of the skeletal system in fetuses

| Indices | Control | Enterosgel doses | | | |
|---|---|---|---|---|---|
| | | 3.21 g/kg (1-6) | 643 mg/kg (6-16) | 3.21 g/kg (6-16) | 3.21 g/kg (16-21) |
| Number of fetuses | 157 | 159 | 153 | 148 | 156 |
| Delayed ossification of the skull bones | 13 | 6 | 2* | 0 | 2* |
| Delayed ossification of the sternum bone / RPPOs | 62 | 30 | 29 | 22* | 22* |
| Number of points ossification of sternum% | 73.4 | 87.4 | 87.4 | 90.1 | 90.1 |
| Shortening the 13th pair of ribs | 6 | 1* | 1* | 3 | 0* |
| The curvature of the ribs | 0 | 0 | 0 | 0 | 0 |
| Number of points ossification<br>metacarpal bone (%)<br>metatarsal bones (%) | <br>91.1<br>91.3 | <br>95.0<br>94.7 | <br>97.4*<br>96.8* | <br>98.2*<br>97.1* | <br>97.0*<br>96.5 * |
| Facial skull Anomaly | 0 | 0 | 0 | 0 | 0 |

**Conclusions**

Enterosgel preparation (paste for oral administration) has no adverse effect on the body of pregnant doe rats, shows no embryotoxic properties, and exerts no negative impact on the development of the offspring.

**Bibliography**

1. Дыбан А.П. Раннее развитие млекопитающих. – Л.: Наука, 1988. – 228 с.
2. Shepard T.H. Catalog Teratogenic Agents. 4th ed. – Baltimore, 1983. – 710 p.
3. Принципы оценки риска для потомства в связи с воздействием химических веществ в период беременности: Совместное издание программы ООН по окружающей среде Международной организации труда и Всемирной организации здравоохранения. – М.: Медицина, 1988. – 155 с.
4. Правила доклинической оценки безопасности фармакологических средств () РД 64–126–91. Москва, 1992.
5. Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ / под ред. Фисенко В.П. – М., ИИА «Ремедиум», 2000. – С. 25–33.
6. Медико-біологічні аспекти застосування ентеросорбенту «Ентеросгель» для лікування різних захворювань (Збірка вибраних наукових статей). – Київ, 2006. – 107 с.
7. Експериментальне вивчення ембріотоксичної дії лікарських засобів (методичні рекомендації) – К. 2000. – 40 с.
8. Staples R.E. et Haseman I.K.Selection of appropriate experimental units in teratology // Teratology. – 1974. – 9(3). – P. 255–259.
9. Wilson J.G. Teratology principles and Techniques.– 1965. – P. 251–277.
10. Лакин Г.Ф. Биометрия: Учебное пособие для биол.спец.вузов. – М.: Высш.шк., 1990. – 352 с.
11. Руководство по клинической лабораторной диагностике Ч.3. // под ред. проф. М.А. Базарновой. – Киев, Вища шк., 1990. – С. 303–311.
12. Лабораторные методы исследования в клинике. Справочник (под ред. Меньшикова В.В.). – М.: Медицина. – 1987. – 350 с.
13. Hossaini A., Dalgard M., Marie A., et al. // Reproductive Toxicology. – 2001, V. 15 (95). – P. 537-543.
14. Kim J.C., Shin H.C., Cha S.W., et al. // Life Scien ces. – 2001, V. 69. – P. 2611–2625