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ORGANOMETRIC CHANGES IN THYMUS UNDER THE INFLUENCE OF PROPYLENE GLYCOL

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Nowadays scientific community is highly attracted by the problem of total environmental pollution, which is becoming more widespread in the period of ever-increasing industrialization and urbanization. According to WHO experts, 80% of human diseases are due to the unfavorable environmental condition due to its contamination by various ecotoxicants [7].

The study of morphofunctional features of the immune system organs and under the influence of various factors of the external and internal environment is a modern and urgent issue of medicine and biology.

The morphological aspects of this problem remain virtually unexplored. Data on morphostructure, linear dimensions and morphometric parameters of the central and peripheral organs of the immune system under conditions of xenobiotic activity almost absent or insufficiently illuminated in the scientific sources. The literature data are fragmentary and singular, therefore, it prompts researchers to study the structure of the thymus under the conditions of xenobiotics compared with the norm [12].

The study of the influence of xenobiotics on immune homeostasis is one of the most pressing problems not only in toxicology, but also in other disciplines. This is due, firstly, to significant pollution of the environment by various compounds that change the immune response (due to the damage to the immunocytes and other blood cells) and cause various diseases; and secondly, the need for correction of immune status changes in the case of chronic intoxication and acute poisoning, accidents at chemical enterprises, in compliance of safety at work, at home, during transportation, storage and destruction of stocks of poisonous substances [6,9].

The scientific direction dealing with the influence of xenobiotics on the non-specific resistance of the organism and the immune system – immunotoxicology – has been formed in the last 30 years. Humanity has created such compounds that are not destroyed in nature under normal conditions – various synthetic polymers, dyes, pesticides, pharmaceuticals, detergents, etc. A huge number of xenobiotics is extremely toxic and shows mutagenic, carcinogenic, allergenic, teratogenic activity [5,15].

Data on the influence of toxicants on the immune system have theoretical value, revealing unknown mechanisms of regulation of immunogenesis, as well as practical, allowing to review the maximum permissible concentrations of various chemical compounds, to conduct scientifically grounded prophylaxis and treatment of acute and chronic intoxications arising from toxic substances of numerous infectious, allergic, autoimmune and cancerous diseases as a result of immune system dysfunction.

Selected for the study of xenobiotic – simple polyester is characterized by fairly significant amounts of synthesis, widely used in various sectors of the economy [17]. Propylene glycol is a part of flavors and food preservatives, used for soaking the poultry, berries, fruits [1].

Materials and methods. Presented research was conducted on 40 WAG matured male rats with initial body weight 180-200 g. White rats were used in the experiment because the structure of their organs of the immune system is not fundamentally different from those of humans. Keeping and manipulation of

animals were carried out in compliance with national and world norms of bioethics [4,10]. Experimental protocols were approved by the ethical committee of Kharkiv National Medical University and Sumy State University.

Animals were randomly divided into two groups. *The first group served as a control (8 animals)* and was kept in conventional environment of vivarium with the usual food and water intake. The second group of rats (32 animals with 8 in each group) was treated via gastric gavage during 7, 15, 30, 45 days by aqueous solutions of PG in doses 1/10 LD₅₀ in conversion to 26,38 g/kg. Intact and experimental rats used in the study were culled decapitated on the day determined by experiment using invented instrument reported in patent [2]. The thymus gland was extracted according to the original method, which has been described in detail in the patent [3]. In compliance with the term of experiment in each group of animals was simultaneously isolated the entire complex of mediastinum with the primary lymphoid organ - thymus. Firstly, the measurements of the organ's linear dimensions using a digital caliper were provided, without its separation, in order to preserve the original topographic anatomical features. The length, width and height of the organ by means of a digital caliper were measured with an accuracy 0.05 mm. Under the width was understood the small axis of the projection of the thymus in the frontal plane. The organs length corresponded to the large axis of the thymus projection in the front plane. Under the height was understood the small axis of the projection of the gland in the sagittal plane. During the experiment, photographing of its individual stages was carried out.

Further, the animal's immune organ was carefully extracted from adipose tissue and weighed using laboratory weights with accuracy 0.25 mg. The volume of organs was calculated using a graduated tube (by displaced fluid volume). The data obtained as a result of morphometric studies, were recorded in protocols, and the mean values were noted into designed individual cards.

To determine the limits of the variability of the morphometric indices of the thymus the method of calculating its indexes using the formulas is used:

$$\text{Ind T} = \frac{W \text{ of thymus}}{L \text{ of thymus}} \times 100; \quad \text{Ind HW} = \frac{H}{W} \times 100; \quad \text{Ind HL} = \frac{H}{L} \times 100,$$

where «W» – width, «L» – length, «H» – height.

For further evaluation of the differences reliability nonparametric criterion of Mann -Whitney was used.

Results and their discussion. Under the influence of PG in a dose of 1/10 LD₅₀, a stable decrease in the thymus mass is observed in comparison to the control group of rats. The thymus mass index varied from min = 180.0×10⁻⁶ kg to max = 310×10⁻⁶ kg, the mean value was from 231.63×10⁻⁶ kg to 258.25×10⁻⁶ kg. Therefore, we have noted the largest change in the weight of the thymus on the 7th and 30th day of the experiment, the mean value of 231.63×10⁻⁶ kg and 240.38×10⁻⁶ kg, respectively. On the 7th day the change in the weight of the thymus was equal to an average of 12.59%, and on 30th day - 21.03% (Table 1).

Table 1. Comparative analysis of rats' thymus mass of control and experimental groups under the influence of PG in a dose 1/10 LD₅₀ (×10⁻⁶ kg)

Indicator	Control group 7th day	Experimental group 7th day	Control group 15th day	Experimental group 15th day	Control group 30th day	Experimental group 30th day	Control group 45th day	Experimental group 45th day
%		12.59		15.25		21.03		10.17
max	310.00	290.00	340.00	290.00	370.00	310.00	320.00	300.00
min	210.00	180.00	220.00	200.00	210.00	190.00	230.00	190.00
mean value	265.00	231.63	287.50	250.13	304.38	240.38	295.13	258.25

It was established that changes in thymus volume of rats on different days of the experiment were similar to those of the thymus mass indexes. Thus, the lowest indicators of thymus volume were also marked on the 7th and 30th days of the experiment and amounted to an average of 229.05×10⁻⁹ m³ and 283.5×10⁻⁹ m³ respectively. It should also be noted that the highest rates of both mass and volume of thymus in the experimental group of rats were on 45th day and amounted to an average of 258.25×10⁻⁶ kg and 312.21×10⁻⁹ m³ respectively. It is established that these parameters on the 45th day of the experiment are most closely related to the minimal parameters of the rats' control group (Fig. 1).

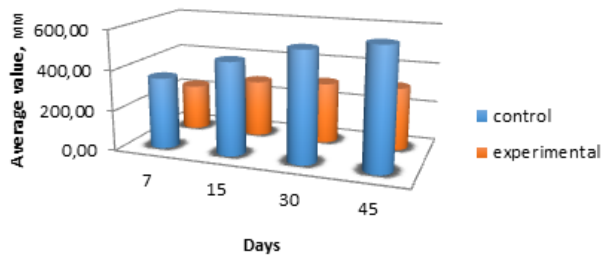


Fig. 1. Indicators of thymus volume in norm and under the influence of PG in a dose of 1/10 LD₅₀ on 7, 15, 30, 45 days of the experiment

The study of the morphometric parameters of the thymus in the experimental group of rats has established a significant reduction of all parameters and their deviation from the parameters of the control group.

Thus, there was a decrease in the thymus width indices throughout the experiment and amounted to a mean value of 12.11×10⁻³ m to 12.86×10⁻³ m. The largest change in the thymus width was the average value of 23.72% for 30th day and 28.17% on the 45th day. These values ranged from min=6.3×10⁻³ m to max=19.5×10⁻³ m. It was also noticed that the smallest percentage change in the thymus width indices occurred on the 7th day and equaled 6.2% (Table 2).

The lowest values of the thymus length were recorded on the 7th and 30th day of the experiment and averaged 14.99×10⁻³ m and 16.31×10⁻³ m, respectively. On 7th day, the change in the thymus length was equal to an average of 14.96%, and on the 30th day, 10.0%. These indicators ranged from min = 12.2×10⁻³ m to max = 19.8×10⁻³ m, the mean value was from 14.99×10⁻³ m to 17.46×10⁻³ m. It was established that these parameters on the 15th and 45th days of the experiment are most closely related to the minimal parameters of the rats' control group - the mean value is 17,19×10⁻³ m and 17,46×10⁻³ m respectively. At the same time, the smallest changes in length are marked on the 45th day of the experiment, which amounted to 8.87% (Table 3).

Table 2. Comparative analysis of rats' thymus width of control and experimental groups under the influence of PG in a dose 1/10 LD₅₀ (×10⁻³ m)

Indicator	Control group 7th day	Experimental group 7th day	Control group 15th day	Experimental group 15th day	Control group 30th day	Experimental group 30th day	Control group 45th day	Experimental group 45th day
%		6.20		18.78		23.72		28.17
max	20.00	18.60	20.70	18.40	20.60	19.50	21.30	18.50
min	8.50	7.20	9.10	6.80	8.90	6.30	9.50	8.60
mean value	13.71	12.86	14.91	12.11	16.18	12.34	16.86	12.11

Table 3. Comparative analysis of rats' thymus length of control group and experimental group under the influence of PG in a dose 1/10 LD₅₀ (×10⁻³ m)

Indicator	Control group 7th day	Experimental group 7th day	Control group 15th day	Experimental group 15th day	Control group 30th day	Experimental group 30th day	Control group 45th day	Experimental group 45th day
%		14.96		12.92		10.00		8.87
max	22.00	18.30	22.50	18.60	21.80	19.30	23.60	19.80
min	14.00	12.20	16.50	14.70	15.70	13.80	14.80	14.20
mean value	17.63	14.99	19.74	17.19	18.13	16.31	19.16	17.46

Table 4. Comparative analysis of rats' thymus height of control group and experimental group under the influence of PG in a dose $1/10 LD_{50}$ ($\times 10^{-3}$ m)

Indicator	Control group 7th day	Experimental group 7th day	Control group 15th day	Experimental group 15th day	Control group 30th day	Experimental group 30th day	Control group 45th day	Experimental group 45th day
%		13.15		5.59		25.76		13.33
max	6.50	5.80	7.20	6.30	7.60	6.80	8.40	6.70
min	2.50	2.30	2.80	2.20	3.20	2.70	3.00	2.30
mean value	4.56	3.96	4.48	4.23	5.78	4.29	5.25	4.55

The lowest indicators of thymus height were noted on the 7th day of the experiment and amounted to an average of 3.96×10^{-3} m. On 7th day, the change in the height of the thymus was equal to an average of 13.15%. These figures ranged from min = 2.2×10^{-3} m to max = 6.8×10^{-3} m, the mean value was from 3.96×10^{-3} m to 4.55×10^{-3} m. It was noted that the reduction of these thymus heights indicators on the 15th, 30th and 45th days of the experiment was almost the same, way below the mean value of the thymus height of the control group. At the same time, among the experimental rats, the greatest approximation of height parameters to the minimal norm indicators among the control group was marked on the 45th day. In addition, for the 30th day, the highest percentage is set - 25.76% of the change in the height of the thymus in the control group (Table 4).

It should also be noted that the highest rates of thymus length and height in the experimental group of rats were on the 45th day and amounted to an average value of 17.46×10^{-9} m³ and a height of 4.55×10^{-9} m³. It is established that these parameters on the 45th day of the experiment are close to the minimal norm of the rats' control group.

Thus, we have found macroscopic and organometric features of the thymus structure under the influence of PG at a dose of $1/10 LD_{50}$, at different stage of the experiment (7, 15, 30, 45 days).

Insignificant influence on the mass indexes on the 7th and 15th days, the width on the 7th and 15th days, the length on the 7th, 15th, 30th and 45th days, on height indices at the 7th, 15th, and 30th days affirmed during the analysis of changes in these indicators.

In studying the peculiarities of the morphometric indices variability of the thymus under the influence of PG at a dose of $1/10 LD_{50}$ we established the following limits of their oscillations: IndHL of the experimental group thymus ranged from min = 12.57 to max = 47.54, the mean value was from 24.67 to 28.02; IndHW of of the experimental group thymus ranged from min=11.96 to max=88.73, the mean value was from 36.78 to 41.41; IndT of the experimental group ranged from min = 38.17 to max = 141.3, the mean value was from 71.1 to 86.52 (Fig. 2-4).

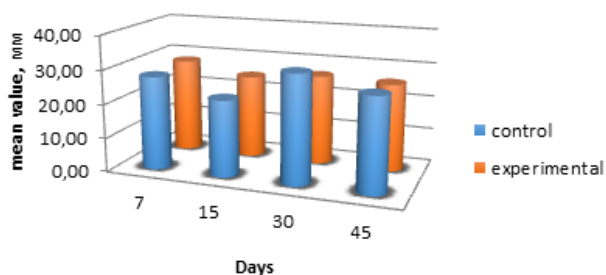


Fig. 2. The indices of IndHL of the rats' thymus of control group and under the influence of PG in a dose of $1/10 LD_{50}$ on 7, 15, 30, 45 days of the experiment

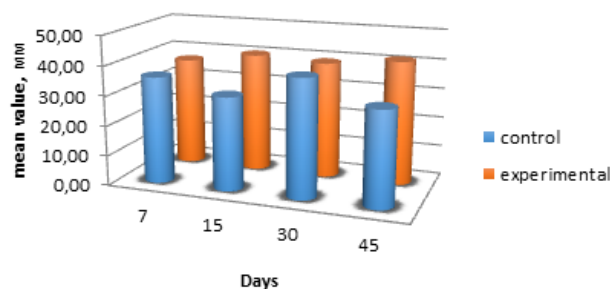


Fig. 3. The indices of IndHW of the rats' thymus of control group and under the influence of PG in a dose of $1/10 LD_{50}$ on 7, 15, 30, 45 days of the experiment

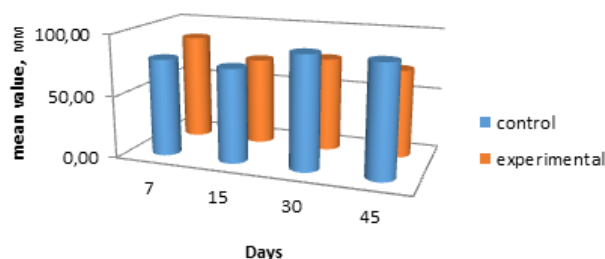


Fig. 4. The indices of IndT of the rats' thymus of control group and under the influence of PG in a dose of $1/10 LD_{50}$ on 7, 15, 30, 45 days of the experiment

Analysis revealed organometric features of the thymus structure under the influence of Propylene glycol in a dose of $1/10 LD_{50}$ at different periods of observation (7, 15, 30, 45 days). Noted maximal decrease of mass and volume on 7th and 30th day which is apparently connected to the active immediate response of the thymus to induced xenobiotic.

In our study, the forms of variability of the thymus under the conditions of xenobiotic from the group of simple polyesters were revealed. Obtained statistical data and derived indices, which can be used in additional instrumental studies (Ultrasound, CT, MRI) to determine the limits of the norm and likely limits of the influence of xenobiotics on the thymus in human were calculated.

Study revealed that IndT of the control group, which is related to the length and width of the thymus, has the greatest limits of the parameters fluctuations and their significant variability. IndHW of the thymus of the control group, which is associated with the height and length of the thymus, has the lowest fluctuation limits of the parameters. In our opinion, this is connected, first of all, with the peculiarities of the structure and form of the rats' thymus.

According to the scientific sources a reliable indicator of local and systemic immunotoxicity evaluation in order to access

the impact of xenobiotic on such complex system as immune multi-stage approach is proposed [16]. Before proceeding to microscopic evaluation of immune organs is reasonable to evaluate mass, volume and linear dimension changes. Discovery of morphological changes in the preclinical stages of the formation of transformations in target organs is an important task for researchers. However, in scientific literature were not found enough works which elucidate the volume measurements of immune organs due to xenobiotics impact which is essential in our opinion for understanding the cellular component of the organ.

After thorough evaluation of scientific literature regarding influence of xenobiotics on organism were not found investigations of propylene glycol impact on the organometric indices of immune organs, thus, we can affirm that our obtained data is new. However, certain authors studied the impact of similar by influence chemicals on organometry of thymus and spleen.

The influence of tartrazine, which is included in many foods preservatives same as propylene glycol was studied by Lysenko SG [11] and showed a decrease in all organometric indices of the thymus during the course of the experiment, but the weight, on the contrary, increased. The data we received do not coincide with these results, the mass of thymus in our research diminished with the experiment time under the conditions of the PG. Under the conditions of 1/10 LD₅₀, the effect on weight and thymus length indices on the 7th, 30th day of the experiment, on the thymus width indices throughout the experiment, on the thymus height indices for the 7th day of the experiment was noted.

In 2012, Kovezhnikov VG [8] studied the effect of sodium glutamate on the thymus's morphogenesis and noticed that it leads to a significant decrease in organometric indices of the organ. These data coincide with those we received in the study, but under the conditions of 1/10 LD₅₀ PG the most reduction of the morphometric indices of the thymus corresponds to the 7th and 45th day.

Study of linear dimensions of thymus by Voloshin VM [17] under the conditions of epichlorohydrin showed that in animals there are phenomena of incidental involution and reduction of all linear parameters and mass during the experiment, but to a lesser extent thymus height. The corresponding data we obtained when studying the effect of 1/100 LD₅₀ PG on thymus and found the highest rates of width and height of thymus in the experimental group of rats were at the 7th and 15th day, and the length of the 7th and 30th day.

Conclusion. 1. The obtained data reveals changes of the thymus linear dimensions, mass and volume under the impact of widely used xenobiotic PG in a dose 1/10 LD₅₀ with the variable response according to the term of experiment.

2. The most significant changes of the mass, volume, length, width, height of organ were noted 7th and 30th days of research, which was due to the active initial reaction of thymus on exogenous impact.

3. The method of indices calculation according to the classical formulas used to determine the variability oscillations of the thymus morphometric parameters was used. It makes practical importance in additional instrumental studies.

4. The fluctuations of the individual variability of the indices of the thymus mass of laboratory rats are established in the norm and due to the PG impact.

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SUMMARY

ORGANOMETRIC CHANGES IN THYMUS UNDER THE INFLUENCE OF PROPYLENE GLYCOL

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The scientific interest in the influence of xenobiotics on the human body is due to the fact that immune organs are characterized by a pronounced response to the influence of endogenous and exogenous factors. Recently, the immunological impairment, as a manifestation of reactions to ecopathogenic conditions, suggests a major pathogenesis role in the development of cardiovascular, neuropsychiatric diseases, as well as diffuse diseases of connective tissue.

Objectives - experiment was designed to elucidate the organometric changes in thymus of male rats due to impact of propylene glycol.

40 WAG matured male rats were divided randomly into two groups. The first group served as a control and constituted 8 animals. The second group of 32 rats, 8 rodents in each, were treated via gavage by aqueous solutions of propylene glycol in dose 1/10 LD₅₀ in conversion to 26,38 g/kg during 7, 15, 30, 45 days. All animals were sacrificed on the term defined by experimental design. Thymus specimens were dissected out, and linear dimensions (length, width, height) using digital caliper were measured, along with mass and volume of the thymus. Limits of the thymus morphometric indices' variability in intact and experimental groups were calculated.

The research indicates that exposure to propylene glycol caused marked organometric changes in rats' thymus. However, more pronounced changes were observed on 7th and 30th days. Were established the following limits of variability indices oscillations: IndHL of the experimental group thymus ranged from min=12.57 to max=47.54, the mean value was from 24.67 to 28.02; IndHW of the experimental group thymus ranged from min=11.96 to max=88.73, the mean value was from 36.78 to 41.41; IndT of the experimental group ranged from min = 38.17 to max=141.3, the mean value was from 71.1 to 86.52. The study of the morphometric parameters of the thymus in the experimental group of rats has established a significant reduction of all parameters and their deviation from the parameters of the control group, that shows active reaction of thymus on induced xenobiotic.

Keywords: Thymus - Rats – Xenobiotics – Organometry – Propylene glycol.

РЕЗЮМЕ

ОРГАНОМЕТРИЧЕСКИЕ ИЗМЕНЕНИЯ ТИМУСА ПОД ВЛИЯНИЕМ ПРОПИЛЕНГЛИКОЛЯ

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Научная заинтересованность влияния ксенобиотиков на организм человека вызвана тем, что иммунным органам

свойственна ярко выраженная ответная реакция на воздействие эндогенных и экзогенных факторов. В последнее время иммунологическим нарушениям, как проявлениям реакций на экопатогенные условия, отводится основная патогенетическая роль в развитии сердечно-сосудистых, нервно-психических заболеваний и диффузных заболеваний соединительной ткани.

Цель исследования - определение органометрических изменений в тимусе самцов крыс под воздействием пропиленгликоля.

40 половозрелых крыс самцов линии WAG были разделены на две группы: I контрольную группу составили 8 животных, II основную группу - 32 крысы, разделенные на 4 подгруппы по 8 грызунов в каждой, которым через желудочный зонд вводили водный раствор пропиленгликоля в дозе 1/10 ДЛ₅₀ в пересчете на 26,38 г/кг в течение 7, 15, 30, 45 дней. После декапитации животных в соответствии со сроком, установленным в эксперименте, извлекался тимус и измерялись линейные размеры (длина, ширина, высота) с помощью цифрового штангенциркуля, а также определялись масса и объем органа. Рассчитаны пределы изменчивости морфометрических показателей тимуса в интактной и экспериментальных группах.

На основании проведенного исследования установлено, что воздействие пропиленгликоля вызывает органометрические изменения в тимусе крыс. Однако более выраженные изменения наблюдались на 7 и 30 сутки. Установлены следующие пределы колебаний индексов индивидуальной изменчивости: **IndHL тимуса в экспериментальной группе** колебался в пределах от min=12,57 до max=47,54, среднее значение составило от 24,67 до 28,02; **IndHW тимуса варьировал** в пределах от min=11,96 до max=88,73, среднее значение - 36,78-41,41; **IndT экспериментальной группы** варьировал в пределах от min=38,17 до max=141,3, среднее значение составило 71,1-86,52.

Изучение морфометрических параметров тимуса в экспериментальной группе крыс позволило установить достоверное снижение всех параметров и их отклонение от показателей контрольной группы, что свидетельствует об активной реакции тимуса на индуцированный ксенобиотик.

რეზიუმე

თიმუსის ორგანომეტრიული ცვლილებები პროპილენგლიკოლის ზემოქმედებით

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სარკოვის ეროვნული სამედიცინო უნივერსიტეტი, ¹ადამიანის ანატომიის კათედრა, ²ბიოქიმიის კათედრა; ³სუმის სახელმწიფო უნივერსიტეტი, მორფოლოგიის კათედრა, უკრაინა

ადამიანის ორგანიზმზე ქსენობიოტიკების გავლენისადმი სამეცნიერო ინტერესი იმითაა პირობადებული, რომ იმუნური ორგანოებისათვის დამახასიათებელია საპასუხო რეაქცია ენდო- და ეგზოგენური ფაქტორების მოქმედებაზე. ბოლო პერიოდში იმუნოლოგიურ დარღვევებს, როგორც რეაქციის გამოვლინებას ეკოპათოგენურ პირობებზე, ძირითადი პათოგენური როლი ენიჭება გულ-სისხლძარღვთა, ნერვულ-ფსიქიკური, ასევე, შემაერთებელი ქსოვილის დიფუზური დაზარალებების განვითარებაში.

კვლევის მიზანს წარმოადგენდა მამრი ვირთაგვების თიმუსის ორგანომეტრიული ცვლილებების განსაზღვრა პროპილენგლიკოლის ზეგავლენის პირობებში. WAG სახის 40 ზრდასრული მამრი ვირთაგვა დაიყო ორ ჯგუფად. პირველი, საკონტროლო ჯგუფი შეადგინა 8 ცხოველმა. მეორე ჯგუფის ცხოველებში (32 ვირთაგვა= 8 ცხოველი ოთხ ქვეჯგუფში) 7, 15, 30 და 45 დღის განმავლობაში კუჭის ზონდის საშუალებით შეჰყავდათ პროპილენგლიკოლის წყალხსნარი, დოზით 1/10 დღე გადანაგარიშებით 26,38 გ/კგ. ექსპერიმენტით დადგენილი ვადის შესაბამისად ცხოველების დეკაპიტაციის შემდეგ, იკვებებოდა თიმუსი; ციფრული შტანგენფარგლის საშუალებით ისაზღვრებოდა მისი ხაზოვანი ზომები (სიგრძე, სიგანე, სიმაღლე), ასევე, ორგანის მასა და მოცულობა. გამოთვლილია მორფომეტრიული მანვენებლების ცვალებადობის საზღვრები ცხოველების ინტაქტურ და ექსპერიმენტულ ჯგუფებში.

ნატარებული კვლევის საფუძველზე დადგენილია, რომ პროპილენგლიკოლის ზემოქმედება იწვევს ორგანომეტრიულ ცვლილებებს ვირთაგვების თიმუსში. თუმცა, უფრო გამოხატული ცვლილებები აღინიშნა მე-7 და 30-ე დღეს. დადგენილია ინდივიდური ცვალებადობის შემდეგი საზღვრები: თიმუსის IndHL ექსპერიმენტულ ჯგუფში მერყეობს min=12,57-დან max=47,54-მდე, საშუალო სიდიდემ შეადგინა 24,67-დან 28,02-მდე; თიმუსის IndHW ექსპერიმენტულ ჯგუფში მერყეობს min=11,96-დან max=88,73-მდე, საშუალო სიდიდემ შეადგინა 36,78-დან 41,41-მდე; IndT ექსპერიმენტულ ჯგუფში მერყეობს min=38,17-დან max=141,3-მდე, საშუალო სიდიდემ შეადგინა 71,1-დან 86,52-მდე. თიმუსის მორფომეტრიული პარამეტრების შესწავლით დადგენილია ყველა პარამეტრის სარწმუნო შემცირება ექსპერიმენტული ჯგუფის ვირთაგვებში და მათი გადახრა საკონტროლო ჯგუფის მონაცემებიდან, რაც ადასტურებს თიმუსის აქტიურ რეაქციას ინდუცირებულ ქსენობიოტიკზე.

PHENOTYPIC CHARACTERISTICS OF TROPHOBLASTIC HYPERPLASIA AND MICROENVIRONMENT ALTERATIONS IN CHORIONIC VILLI IN SPONTANEOUS ABORTIONS

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The incidence of 15-20% clinical pregnancies end up with spontaneous abortion, and 85% of spontaneous abortions are developed during first trimester of pregnancy, before 20th gestational week [1]. Therefore, all pregnant women are under 15% risk of the development spontaneous abortion. Frequently, the reason of spontaneous abortion in first trimester is unknown. Approximately 50% of spontaneous abortions are caused by different foetal chromosomal abnormalities, such as aneuploidy, polyploidy and different types of translocations. In rest of the cases, spontaneous abortion might be caused by different maternal causes, such as pathology of uterus and placenta and endocrine and immune disturbances, as well as by infectious agents [2]. Risk factors of spontaneous abortions include: age >30, smoking, alcohol consumption and high number of deliveries. Precise identification of the reasons of spontaneous abortion is important, first of all in order to avoid complications and second, in order to plan next pregnancy correctly [3]. In 2015 the Institute for development of freedom information (IDFI) published an abortion statistic, according to which the number of spontaneous abortions significantly increased in Georgia from 2004 to 2014. Particularly, the number of registered spontaneous abortions was 2224 in the year of 2004, whilst it reached 5895 cases by the year of 2014. The current statistics of spontaneous abortions is unknown, as well as there is no information about the causative factors of spontaneous abortions in Georgia.

Molar pregnancy, represents a special cause of spontaneous abortions and deserves further attention due to the difficulty of differential diagnosis amongst other causes [4]. At the beginning, molar pregnancy resembles to normal pregnancy, however already in first trimester vaginal bleeding and spontaneous abortion develops [5]. The major cause of molar pregnancy is hydatidiform mole. Two major types of hydatidiform moles

can be differentiated, such as partial mole and complete mole. They develop due to pathological fertilisation. In case of partial mole, normal oocyte is fertilised by two sperms, and triploid zygote with 69XX karyotype develops. Complete moles are characterised with 46XX karyotype of paternal origin, due to fertilisation of empty oocyte, which subsequently undergoes duplication. Hence, partial mole contains one maternal and two paternal genomes, whilst complete mole contains only paternal genome [6,7]. Hydatidiform moles are characterised with abnormal hyperplasia of trophoblast and hydropic swelling of chorionic villi. Invasive mole represent the malignant counterpart of hydatidiform mole, which is also caused by pathology of fertilisation and it is characterised with local invasion of mole [8].

Chorionic villi are covered by two types of epithelial cell layers, syncytiotrophoblast and cytotrophoblast, from which major layer syncytiotrophoblast lines the intervillous space and it is in direct contact with maternal blood. Underlying stromal cells are located adjacent to foetal capillaries and are mainly composed of fibroblasts and so called Hofbauer cells (HBCs), which represent the CD68 positive foetal tissue macrophages [9]. In addition to macrophages, different subpopulations of other immune cells can be found in placental tissue. The study from Fasial et al., demonstrated that the number of CD3 and CD8 positive T cells are increased in case of molar pregnancies, compared to normal placenta [10].

Differential diagnosis between complete and partial mole, using standard diagnostic, haematoxylin and eosin (H&E) stained, as well as by cytogenetic analysis is less reliable. Also, there is no histological parameters known, which indicates the invasive potential of hydatidiform moles and additional immunohistochemical investigation is recommended. However, the immunohistochemical features of trophoblastic hyperplasia is not well