The interconnection of the elemental composition and morphofunctional characteristics of placenta during the tobacco intoxication

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Abstract. The aim of the research was to study the interconnection between the elemental composition and morphofunctional characteristics of the rat placenta during the tobacco intoxication. Determining the elemental composition of the placenta was performed using an Elan 9000 mass spectrometer and an Optima 2000 V atomic emission spectrometer. The morphofunctional characteristics of the placenta were studied by preparing the serial paraffin histological sections. Data processing was performed using the Mann-Whitney U-test. It was established that as the result of a passive smoking, the fertility of females decreased, the physical development of fetuses also decreased, the weight and thickness of the placenta declined, the number of fetal capillaries and maternal sinuses of the placenta decreased. The most common pathological changes in the histological structure of the placentas of the experimental groups were the development of foci of circulatory disorders, inflammatory and atrophic changes. When comparing the elemental composition of the experimental groups, lower values of magnesium and iron were found, against the background of higher values of lead and cadmium. Identified dislementasis in conjunction with the pathological changes in the placenta caused a placental insufficiency, which in turn led to the delay in fetal development of the fetus.

1. Introduction
Despite the expansion of the tobacco control measures, smoking continues to be one of the leading risk factors for death and disability worldwide [1]. The prevalence of smoking among women in Russia increased from 5% in the 1980s to 20% in the 2000s [2]. The problem of passive smoking is no less dangerous [3]. It has been established that with the passive smoking, it is possible to increase the level of nicotine in the blood plasma, which is equivalent to the level of regular smoking of tobacco [4]. According to the statistics, more than a third of women aged 15 years and older are regularly subjected to the secondary tobacco smoke [5]. Cigarette smoke, both primary and secondary, contains thousands of components, including carcinogens and cytotoxic chemicals [8]. The effect of passive smoking correlates with the increase in toxic elements, which are antagonists of a number of essential macro- and microelements [6, 7]. Smoking is a significant factor that affects not only the reproduction and the process of pregnancy, but also the offspring. The danger of tobacco smoke is that it has an effect on reducing the duration of pregnancy, the weight of the newborn and the structure of the placenta [9]. In the large-scale epidemiological studies, it was revealed that smoking during the pregnancy may increase
the risk of rejection of the placenta, as well as its law insertion [10, 11]. Modern research confirms that the passive smoking during the pregnancy can have a negative effect on the body of the mother and the unborn child, therefore, an understanding of these mechanisms is central to ensuring a healthy pregnancy and the development of the child.

2. Purpose of research
To identify the interconnection between the elemental composition and morphofunctional characteristics of the rat placenta with the tobacco intoxication.

3. Materials and methods
Experimental studies were carried out in accordance with the provisions of the Helsinki Declaration concerning the humane treatment of animals. Investigations were carried out in the experimental biological clinic on 20 mature doe Wistar rats, the two groups were formed from their - control and experimental. The exposure of doe rats in the atmosphere of tobacco smoke was carried out before and after pregnancy. For 3 weeks, non-pregnant rats of the experimental group were exposed daily (2 times a day) it was a 30-minute exposure of tobacco smoke in the exposure chamber. At the end of this period males were added to the groups of does to produce the offspring. After the establishment of pregnancy, the exposure of tobacco smoke lasted another 3 weeks. Each rat of the experimental group received a maximum of 0.048 mg of nicotine, which is equivalent to the dose of the average smoker. The control group throughout the experiment was 2 times a day for 30 minutes in the exposure chamber in the absence of a damaging factor. For research of the placental complex, the selected material was fixed in 10% neutral formalin with the subsequent preparation of serial paraffin histological sections, which after dewaxing were stained with the hematoxylin and eosin. Determination of the elemental composition of the placenta was carried out in the laboratory of the ANCO "Center for Biotic Medicine" at the ICP-AES and ICP-MS. The statistical processing of digital material was carried out using the application "Excel" and "Statistica 6.0". The statistical significance of differences between the groups was assessed using the Mann-Whitney U-test.

4. Results
Analyzing the data obtained during the experiment, the fertility of rats in the experimental group was found to decrease by 14.1% (p≤0.05) as compared to the control group, the average weight decreased by 14.9% (p≤0.05) and the length of the fetus - by 22.4% (p≤0.05). In addition to changing the number of fetuses and their physical development, 4 dead fetuses were found in the group under the influence of tobacco smoke. Changes in the morphometric parameters of the placenta of the experimental group were detected, a decrease in the mass of the placenta by 5.2% (p ≤ 0.05) and a thickness by 8.4%, it is relative to the control group. There was a decrease in the number of fetus capillaries by 14.3% (p <0.05) and maternal sinuses by 23.4% (p <0.01) with a general tendency for a decrease in their diameters.

Exposure of the tobacco smoke had an effect on the histological structure of the placenta (Figure 1). In the placentas of animals of the experimental groups, an uneven thickening of the chorionic plate of the fetal part was observed, and dilated thick-walled blood vessels with uneven fullness also were revealed. Minor hemorrhages and increased cellular infiltration were noted with the further transition to the perivascular tissue. Circulatory disorders in most cases were accompanied by the structural and functional activation of the parietal trophoblast, which was expressed in the uneven focal hyperplasia of the giant cells and the appearance of their multi-core forms. The trophoblast layer had an uneven thickness, signs of pyknosis, nuclear lysis and cytoplasm vacuolization, large foci of cell destruction with the formation of different cavity sizes (Figure 16). The number of layers increased from 2-3 in the control group to 6-8 in the experimental group.

An increase in the area of the spongy extra-labyrinth layer with the randomly located glycogenic pockets, which had a disturbed structure, was found in the placenta of animals with the tobacco intoxication. The labyrinth layer of the placenta, the main structures of which are the fetus capillaries and maternal lacunae, had a number of pathological changes in the experimental group: a sharp plethora, swelling or hemorrhage (Figure 1в). Foci of ischemia and thrombosis of maternal lacunae were often seen. Dystrophic changes developed in the trophoblast cells.
When comparing the elemental composition of the placentas of the experimental and control groups, a number of changes were revealed (Table 1).

**Table 1.** Concentrations of trace elements in placenta of rats, Me (q25 – q75), (mg/kg)

| Indicators | Groups          | Control                  | Experience               |
|------------|-----------------|--------------------------|--------------------------|
| Ca         |                 | 104 (90-114)             | 114.3 (95-131)           |
| P          |                 | 1624 (1402-1786)         | 1391 (1257-1537)         |
| Mg         |                 | 134 (118-147)            | 73.4 (60.8-85)\(a\)      |
| Na         |                 | 1457 (1300-1603)         | 1435 (1259-1539)         |
| K          |                 | 2086 (1807-2295)         | 2040 (1838-2246)         |
| Fe         |                 | 152 (142-162)            | 116.3 (104-131)\(b\)     |
| As         |                 | 0.11 (0.089-0.125)       | 0.08 (0.066-0.11)        |
| Si         |                 | 41.7 (37.3-45.8)         | 33.1 (26.8-39.6)         |
| Zn         |                 | 18.1 (16.3-19.9)         | 15.6 (14.2-17.4)         |
| Cu         |                 | 2.1 (1.86-2.28)          | 2.4 (2.14-2.82)          |
| I          |                 | 0.16 (0.14-0.18)         | 0.18 (0.16-0.19)         |
| Cd         |                 | 0.00115 (0.001-0.0014)   | 0.006 (0.005-0.007)\(c\) |
| Pb         |                 | 0.019 (0.016-0.028)      | 0.031 (0.026-0.039)\(b\) |

\(a\) Reliability of differences in performance with the control group – \(p<0.05\)

\(b\) Reliability of differences in performance with the control group – \(p<0.01\)

\(c\) Reliability of differences in performance with the control group – \(p<0.001\)

In the experimental group, the level of magnesium was reliably lower by 45.2% (\(p\leq0.05\)). There was a tendency to the lower values of other macronutrients, phosphorus by 14.4%, sodium by 1.6% and potassium by 2.3%. Calcium was the exception, the concentration of which was higher by 9.03%. When comparing the content of essential and conditionally essential elements, reliable differences were obtained only for iron, the content of which was lower by 23.4% (\(p <0.01\)) in the experimental group. A trend towards the lower values of silicon and zinc was recorded by 21% and by 14%, respectively. At the same time, the content of copper and iodine increased by 14.4%, 12.4%, respectively. The accumulation of toxic elements in the placenta of pregnant rats seems natural. In the studied samples of the experimental group, there was a significant increase of the content of cadmium by 79.8% (\(p <0.001\))
and lead - by 63.2% (p <0.05). With respect to another chemical elements, no statistically significant differences were found.

5. Discussion

The proper placental development is central to the health of both the mother during the pregnancy and the fetus. Analyzing the data obtained in the experiment, it can be noted that passive smoking influenced the number and physical development of the fetus. It is known that tobacco changes the mitochondrial respiratory function in cardiomyocytes and lung tissue, for this reason it is hypothesized that the placental mitochondrial function can also be changed as a result of smoking [13]. In this regard, the results show the possibility of the development of placental mitochondrial dysfunction, which could help limit the fetal growth by limiting the availability of energy in the cells. On the other hand, researchers from Turkey suggest that thiocyanat coming from the cigarettes can inhibit the iodine transfer in the placenta and thus adversely affect the fetal development through this mechanism [14].

The most important component of the normal functioning of the placenta is the proper development of its vascular system [12]. The revealed statistically significant decrease in the number of fetus capillaries and maternal sinuses disrupts the blood supply to the placenta, which is also a factor limiting the fetal growth and in some cases leading to the fetal death. The most common pathological changes in the histological structure of the placentas of the experimental groups were the development of foci of circulatory disorders, inflammatory and atrophic changes. The above-mentioned histology results indicate that the slow growth of the fetus is due to the impaired uteroplacental circulation as a result of the damaging effects of nicotine. Thus, the obtained histological data suggest that maternal smoking can affect the health of the fetus, changing the structure of the placenta, and, consequently, its function [15].

Analyzing the data on the elemental composition of the placenta, it was revealed that during the passive smoking there are low values of the majority of vital elements and high values of toxic elements in the placenta. The detected magnesium deficiency, presumably, as well as its excess can inhibit the work of caspase-3, which is necessary for the normal functioning of the placenta [16]. The observed low iron content is dangerous for the unborn child, since the intrauterine amount of this element is important for the development of the fetus and helps to create the iron reserves, sufficient to maintain the growth in early infancy [17, 23]. Among all the macronutrients, only calcium had a higher content. It has been established that smoking is one of the factors that contributes to the formation of calcium deposits in the placenta [18]. Due to the placenta calcification, the fetus may not receive enough nutrients and oxygen, which can later cause a number of pathologies of the child, and in some cases lead to the stillbirth [19].

There was a significant increase in the concentrations of cadmium and lead in the placenta of rats with tobacco intoxication. Cadmium and other metal ions can act as the metal estrogens and endocrine tissue disrupters and impair the fetal development in mammals [20]. Negative effects occur in relation to the synthesis and release of leptin. Leptin is produced by the trophoblast and can regulate the organogenesis and fetal development. It is believed that cadmium can lead to the decrease in the synthesis of placental leptin, which in turn impairs the formation of placental progesterone. Such violations are further evidence of the endocrine influence of cadmium and lead, as the components of the tobacco smoke, on the reproductive capacity of women. The group of Chinese scientists found that smoking cigarettes by pregnant women increases the accumulation of cadmium in the placental tissue and it is possible that this element has a stimulating effect on the production of placenta metallothionein [22]. Elevated concentrations of cadmium and lead in the placenta of pregnant women increase the risk of spontaneous abortion [21]. Therefore, the levels of these heavy metals can be used as additional markers to the already existing diagnostic procedures, especially in the early stages of pregnancy, as the modern tools in perinatal care, to allow early diagnosis of pregnancy pathology and, especially, to prevent the spontaneous abortions [23, 24].

6. Conclusion

Taking into account the changes observed in the placentas of rats, it can be assumed that the identified disease elementosis, together with the pathological changes in the placenta, is the cause of the placental insufficiency among the pregnant doe rats. The placental insufficiency provoked by the exposure of
tobacco smoke led to the delay in the fetal development and fetal death among the experimental animals as a result of a violation of the trophic, transport, metabolic and other functions of the placenta.

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References
[1] Reitsma M B, Fullman N, Salama J S et al 2017 Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015 *Lancet* 13 pp 1885–906
[2] Kharkova O, Krettek A, Grijbovski A, Nieboer E and Odland O 2016 Prevalence of smoking before and during pregnancy and changes in this habit during pregnancy in Northwest Russia: a Murmansk county birth registry study *Reproductive Health*. 18
[3] Yan H Zh, Yim W M, Grace W and Ho K 2019 Effectiveness of Interventions to Reduce Exposure to Parental Secondhand Smoke at Home among Children in China: A Systematic Review *Int. J. of Environmental Res. and Public Health* 1(16)
[4] Okoli C T, Kelly T and Hahn E J 2007 Secondhand smoke and nicotine exposure: a brief review *Addictive Behaviors* 32(10) 1977–88
[5] Kuzina O A, Vasilevskaya G V and Avdeeva M E 2016 About relevance of passive smoking during pregnancy *Interactive sci*. 142–3
[6] Yang L, Tong E K, Mao Z and Hu T W 2010 Exposure to secondhand smoke and associated factors among non-smoking pregnant women with smoking husbands in Sichuan province, China *Acta Obstetricia et Gynecologica Scandinavica* 89(4) 549–57
[7] Dursun A, Yurdakok K, Yalcin S S, Tekinalp G, Aykut O, Orhan G and Morgil G K 2016 Maternal risk factors associated with lead, mercury and cadmium levels in umbilical cord blood, breast milk and newborn hair *Journal of Maternal-Fetal and Neonatal Medicine* 29(6) 954–61
[8] Lewis J B, Hirschi K M, Arroyo JA, Bikman B T, Kooyman D L and Reynolds P R 2017 Plausible Roles for RAGE in Conditions Exacerbated by Direct and Indirect (Secondhand) Smoke Exposure *Int. J. of Molecular Sci*. 18(3)
[9] Urbaniai T, Klejewski A and Sobczyk K 2015 Influence of smoking on pregnancy course and fetal development *Przegląd lekarski* 72(3) 144–7
[10] Shobeiri F, Masoumi S Z and Jenabi E 2017 The association between maternal smoking and placenta abruption: a meta-analysis *J. of Maternal-Fetal and Neonatal Medicine* 30(16) 1963–7
[11] Shobeiri F and Jenabi E 2017 Smoking and placenta previa: a meta-analysis *J. of Maternal-Fetal and Neonatal Medicine* 30(24) 2985–90
[12] Pereira R D, De Long N E, Wang R C, Yazdi F T, Holloway A C and Raha S 2015 Angiogenesis in the placenta: the role of reactive oxygen species signaling *BioMed Res. Int.*
[13] Bouhours-Nouet N, May-Panloup P, Coutant R, de Casson F B, Descamps P, Douay O, Reynier P, Ritz P, Malthiéry Y and Simard G 2005 Maternal smoking is associated with mitochondrial DNA depletion and respiratory chain complex III deficiency in placenta *AJP Endocrinology and Metabolism* 288(1) 171–7
[14] Lebedev S, Gavrish I A, Gubaydullina I Z 2019 Different chrome sources influence on morphobiochemical indicators and activity of digestive enzymes in wistar rats *Sel'skokhozvaistvennaya biologiya* [Agricultural Biology] 54 (2) 304-315
[15] Heidari Z, Mahmoudzadeh-Sagheb H and Sheibak N 2018 Quantitative changes of extravillous trophoblast cells in heavy smoker mothers compared with healthy controls *Reproduction Fertility And Development* 30(2) 409–14
[16] Gao H and Zou L 2006 Effect of magnesium sulfate on fetal rats of fetal growth retardation and its relation with expression of caspase-3 on the placenta of maternal rat *Zhonghua Fu Chan Ke Za Zhi* 4(8) 525–8
[17] Cao C and Fleming M D 2016 The placenta: the forgotten essential organ of iron transport *Nutrition Reviews* 74(7) 421–31

[18] Szymanowski K, Chmaj-Wierzchowska K, Florek E and Opala T 2007 Do calcification of placenta reveal only maternal cigarette smoking? *Przegląd lekarski* 64(10) 879–8

[19] Kvan O, Gavrish I, Lebedev S, Korotkova A, Miroshnikova E, Bykov A, Serdaeva V, Davydova N. 2018 Effect of probiotics on the basis of Bacillus subtilis and Bifidobacterium longum on the biochemical parameters of the animal organism. *Environmental Science and Pollution Research* 25(3) 2175-2183

[20] Stasenko S, Bradford E M, Piasek M, Henson M C, Varnai V M, Jurasović J and Kusec V 2010 Metals in human placenta: focus on the effects of cadmium on steroid hormones and leptin *J. of Applied Toxicology* 30(3) 242–53

[21] Omeljaniuk WJ, Socha K, Soroczynska J, Charkiewicz A E, Laudanski T, Kulikowski M, Kobylec E and Borawska M H 2010 Cadmium and Lead in Women WHO Miscarried *Clinical Laboratory* 64(1) 59–67

[22] Ronco A M, Arguello G, Suazo M and Llanos M N 2005 Increased levels of metallothionein in placenta of smokers *Toxicology* 208(1) 133–9

[23] Notova S, Lizurchik L, Kiyaeva E, Larjushina I, Marshinskaya O and Kazakova T 2018 Influence of passive smoking on some biochemical characteristics of amniotic fluid *FEBS Open Bio* 8 229

[24] Lebedev S, Gavrish I, Rusakova E, Kvan O and Gubaidullina I 2018 Influence of various chromium compounds on physiological, morpho-biochemical parameters, and digestive enzymes activity in Wistar rats *Trace elements and electrolytes* 35(4) 242-245