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HISTOSTRUCTURE OF THE THYROID GLAND IN THE SEXUALLY MATURE OFFSPRINGS OF FEMALE RATS WHICH WERE EXPOSED TO PASSIVE SMOKING DURING PREGNANCY

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Abstract

Active smoking or passive entry of tobacco smoke into the body of a pregnant leads to the effects of nicotine on the fetus's cells, which causes many problems of fetal development and health problems in the later life of the offspring. The researches made has conclusively proven that smoking during pregnancy contributes to the development of many endocrine disorders. The effect of tobacco smoke on the increase of leptin, C-reactive protein, decrease of adiponectin, as well as on the appearance of antibodies to islet cells of the pancreas, as the first step to the development of type 1 diabetes, was determined by epidemiological studies. In studying the effects of nicotine during lactation it was concluded that the consumption of 6 mg / kg / day of nicotine, hyperleptinemia and primary hypothyroidism develops in the newborn, and later in adulthood, all components of the metabolic syndrome. At the same time, the data on the effect of nicotine entering the mother's body and its further influence on the offspring's thyroid gland are few. **The objective:** to the study histofunctional and morphometric features of the thyroid gland of both sexes offsprings born by mothers which were exposed to "passive" smoking during pregnancy. **The study object:** 6-month-old Wistar rats, first-generation offspring derived from control (intact) females that were exposed to

tobacco smoke components in a reproduction model of "passive" smoking. Histological and morphometric changes of the thyroid gland of 6-month-old offspring of mothers in whose bodies the products of cigarette combustion entered through the respiratory tract were studied. Morphofunctional criteria of instability and changes in the microstructure of the thyroid gland of offsprings whose mothers were exposed to nicotine in postnatal life were determined. Primary apoptotic-destructive processes in parenchyma, the formation of unstructured cell fields and microfollicles allow us to consider these offsprings as a risk group for thyroid pathology in terms of additional exposure to negative factors of "passive" smoking.

Key words: "passive" smoking of pregnant women; offspring; thyroid gland; morphofunctional features.

Relationship of the publication with the planned research works. The work presented is a fragment of the research project "Study of mothers' "passive smoking" effects during pregnancy on the somato-endocrine phenotype of offsprings (experimental study)", № state registration 0117U007187.

Introduction. It is now well known that tobacco smoke contains 4800 derivatives, of which at least 200 cause endocrine disorders [1]. For example, thiocyanate, cadmium salts, lithium, tobacco resins inhibit various stages of iodine metabolism in the thyroid gland (TG), reduce the activity of key enzymes of thyroid hormonal genesis and metabolism [2]. The negative effect of tobacco smoke on the fetus is almost the same as during active or passive smoking [3].

Active smoking or passive entry of tobacco smoke into the body of a pregnant leads to the effects of nicotine on the fetus's cells, which causes many problems of fetal development and health problems in the later life of the child. It has been proven that smoking reduces body weight at birth by 13 g when using one cigarette per day, and by 533 g when using more than 20 cigarettes and leads to the birth of a child with intrauterine growth retardation [4]. Adverse effects of smoking are not limited to the bad habit of mothers; "passive" smoking has also been shown to pose significant risks to pregnant women and their fetuses [3].

A number of researches has conclusively proven that smoking during pregnancy contributes to the development of many endocrine disorders [5]. It is known that due to the occurrence of inadequate lipid metabolism and lipogenesis, the development of obesity in the body of offspring in childhood and adolescence [6, 7] takes place. Thus, in the study of metabolic syndrome parameters, such as hypertriglyceridemia, central obesity, elevated

fasting insulin, the relationship with maternal smoking was determined [7]. The effect of tobacco smoke on the increase of leptin, C-reactive protein, decrease of adiponectin, as well as on the appearance of antibodies to islet cells of the pancreas as the first step to the development of type 1 diabetes was determined by epidemiological research [8]. The conclusions of clinical and epidemiological observations are confirmed by the results of experimental works [9]. Thus, "passive" smoking, simulated in pregnant female rats, increases fat deposits in the fetus on the 20th day of pregnancy [10]. Adult offspring of such mothers also show insulin resistance and glucose intolerance, high sensitivity to cold, decreased physical activity and a high risk of cardiovascular disease [11]. The researchers have studied the effects of nicotine during lactation and concluded that the consumption of 6 mg / kg / day of nicotine develops hyperleptinemia and primary hypothyroidism in a newborn, and also all components of the metabolic syndrome [12] later in adulthood,. It is also known that in the offspring of smoking mothers, the adrenal glands synthesize catecholamines, but secrete them less (due to the tension of the hypothalamic-pituitary-adrenal system), which contributes to the development of obesity [13].

At the same time, the data on the effect of nicotine entering the mother's body on TG of offspring are very few.

The objective: Taking into account the absence in the literature of characteristic age features of the histostructure of intact rats TG, and even more so, changes inherent in the body under the influence of negative factors during pregnancy, the objective of this study was to highlight the microstructure and morphometric parameters of the both sexes first generation in puberty after exposure to tobacco smoke on a pregnant female throughout gestation.

Object and methods of research. Experimental research was conducted in compliance with the main provisions of the Resolution of the First National Congress on Bioethics "General Ethical Principles of Animal Experiments" (2001), the requirements of humane treatment of experimental animals regulated by the Law of Ukraine "On Protection of Animals from Cruelty" (N-3447- IV dated 21.02.2006) and the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 18.03.1986).

The object of the study were 6-month-old rats of both sexes of the Wistar population - offsprings of the first generation, obtained from control (intact) females (gr.1) and females rats which were exposed to tobacco smoke during the reproduction of "passive" smoking [13] model with low birth weight (gr.2) and normal birth weight (gr.3).

Simulation of "passive" smoking situation was achieved by keeping pregnant female

rats (one animal at a time) in a transparent chamber measuring (95 x 80 x 65) cm³ (volume 0.49 m³), which were located in a separate room at a temperature of 23⁰ C ± 1⁰ C and relative humidity of 50% -60%. Cigarettes were placed in the upper part of the chamber in a special hole that did not allow rats to touch them. Cigarettes were burned completely. Pregnant rats were exposed to nicotine and a complex of other substances during the decay of one cigarette (8-10 minutes) daily throughout pregnancy. Used cigarettes of a commercial brand of the following composition: 0.7 mg of nicotine, 11 mg of tar, which are considered cigarettes of "medium strength". The calculation of the equivalent dose of nicotine and the time of exposure of animals to tobacco smoke was performed on the basis of a tested model [13], according to which one animal received no more than 0.043 mg of nicotine in the experiment.

For histological examination, TG was removed, fixed in a 10% solution of neutral formalin, dehydrated in alcohol, poured into paraffin, made serial sections with a thickness of (4-5) µm. Histological specimens stained with hematoxylin and eosin were used for general assessment of the condition of tissues in the genitals. The study and photography of micropreparations were performed using a "Primo Star" light microscope (Carl Zeiss, Germany) with a built-in Canon G 10 camera. In this case, from one organ of each animal at least 15 serial sections was made, on which from 30 to 70 fields of view were studied. Microscopic analysis of the histostructure, using different magnifications of the microscope was performed.

Morphometry was performed with the use of the computer program Photoshop CS5. Statistical analysis of the data obtained was performed with the statistical software package Excel 2010 and Statistics 6.0. The difference was considered statistically significant at p <0,05.

Results and their discussion. Examination of the 1st group both sexes 6-month-old rats TG microstructure revealed that the parenchyma of the gland is represented by formed follicles, which have an oval-round, and sometimes elongated or irregular shape. The walls of the follicles are formed by thyrocytes of cubic and cylindrical shape, in most cases of the same height in one follicle, due to which the internal boundaries of the follicle cavity are clearly delineated. The basement membranes of the follicles, which include the thinnest layer of connective tissue and fibrocytes, in most cases are clearly expressed.

Meanwhile, the cell membranes of thyrocytes are poorly stained. The cytoplasm of thyroid epithelial cells is weakly oxyphilic. Round, slightly granular nuclei are located in the center of the cell, or are closer to its outer membrane. There are light areas in the cytoplasm of thyrocytes, which indicate an active process of transcellular excretion of thyroxine (T₄) and

thyroglobulin (TGI) into the bloodstream of the interfollicular epithelium a little. In some females, a microfollicular structure was observed, sometimes abundantly infiltrated with lymphocytes.

The histological structure of the thyroid gland of the offspring of experimental rats has certain features that depend on sex and body weight at birth. Thus, in male offspring of 6 months of age group 2, the area of individual lobes of the parenchyma increases; the lobes consist of a much larger number of follicles. The follicles are 2-5 times larger than the corresponding intact animals.

Follicles are mostly of medium size, and large ones are absent, even on the periphery of the gland. Microfollicles generally have a normal structure. Sometimes there is intrafollicular ingrowth of thyrocytes. Interfollicular epithelium is small. In some places you can see violations of the basement membrane of follicles and small foci of lymphoid infiltration.

It should be noted that in males gr.3, in the thyroid gland, along with medium and small follicles, there is a significant amount of interfollicular epithelium infiltrated by lymphocytes. Similar changes in the parenchyma are characteristic of 6-month-old females of the same group, but in these animals the interfollicular epithelium is even greater, its lymphocytic infiltration is more abundant, and the growth of the stroma is more pronounced.

In group 2 6-month-old females, small follicles predominate, sometimes there is intrafollicular growth. At the same time there are large areas of undifferentiated epithelium, which make up almost the whole lobe. In the interfollicular layers are large areas of destructively altered and dead follicles. In the same places abundant lymphoid infiltration is observed. The follicles that have preserved the structure show signs of functional stagnation and intrafollicular ingrowth of thyrocytes.

Males of group 3 are dominated by follicles of medium size, although they are few, but under the capsule there are large follicles. The epithelium of the follicles is swollen, cubic or cylindrical. The density of the colloid is insignificant, which indicates the active functioning of the gland. Quite large areas of undifferentiated epithelium are often found. As lymphocytic infiltration is observed in such places, it is possible to conclude about active local degradation of follicles, resorption of thyroglobulin and death of thyrocytes. The stroma is very developed - sometimes it is whole fields of fibrous connective tissue.

In addition, examination of histological sections of the thyroid gland of a certain part (40%) of gr. 3 females indicates increased development of stromal elements and small-lobed structure of the gland. The parenchyma of the gland is represented by medium-sized follicles.

In these follicles, the epithelium is functionally active and the colloid is liquid.

Fields of interfollicular epithelium are large. Intrafollicular ingrowth of thyrocytes forming epithelial papillae is often observed. The larger area of the parenchyma does not have a typical organization, but is filled with small thyrocytes with compacted nuclei. The space between the follicles is filled with lymphoid infiltration. The stroma is also very developed in many places.

Morphometric examination showed that in 6-month-old offspring (gr.2) diameter of the nucleus of the thyroid epithelium in comparison with gr. 1 and 3 was lower by 19.00% and 14.49%, respectively (5.37 μm vs. 6.63 μm , $p < 0.01$; 5.37 μm vs. 6.28 μm , $p < 0.01$, respectively) .

The average outer diameter of the follicles in gr. 2 in comparison with gr. 1 and 3 was lower by 14.50% and higher by 19.22%, respectively (67.30 μm vs. 78.71 μm and 56.45 μm , $p < 0.01$, respectively). Also in gr. 3 the average outer diameter of the follicles was lower by 28.27% compared with gr. 1 (56.45 μm vs. 78.71 μm , $p < 0.01$).

In gr. 3 the height of the follicular epithelium was lower by 10.18% compared with gr. 1 (12.44 μm vs. 13.85 μm , $p < 0.05$).

Nuclear cytoplasmic index in gr. 2 in comparison with gr. 1 and 3 was higher by 40% (0.014 RU vs. 0.010 RU, $p < 0.05$).

The relative volume of the stroma in gr. 2 was higher by 23.17% compared with gr. 1 (30.30% vs. 24.60%, $p < 0.05$). The relative volume of the vascular bed in gr. 2 was lower by 26.11% compared with gr. 1 (11.60% vs. 15.70%, $p < 0.01$).

In gr. 3, the relative volume of the stroma and the relative volume of the vascular bed did not have statistically significant differences compared with gr. 1 and 2. Also in gr. 2 area of follicles, area of nuclei, relative volume of follicular epithelium and relative volume of interfollicular epithelium had no statistically significant differences when compared with gr. 1 and 3.

Morphometric parameters of the thyroid gland of 6-month-old intact and experimental animals are given in Table 1.

Thus, for most structural indicators gr. 3 differentiated greatly from gr. 1, and in gr. 3 changes of only some structural characteristics were observed.

Table 1

Morphometric parameters of thyroid glands of 6-month-old offspring from intact females and females exposed to passive smoking and restricted nutrition

Morphometric parameters, \bar{X} (s)	Group 1, n = 5	Group 2, n=5	Group 3, n=5
The area of the follicle, μm^2	3992.19 (94.09)	3661.26(86.29) >0.05 ¹⁾	3899.88(91.92) >0.05 ²⁾ >0.05 ³⁾
Core area, μm^2	53.27(1.25)	50.74(1.19) >0.05 ¹⁾	51.96(1.22) >0.05 ²⁾ >0.05 ³⁾
Average outer diameter of follicle, μm	78.71(1.85)	67.30(1.58) <0.01 ¹⁾	56.45(1.33) <0.01 ²⁾ <0.01 ³⁾
Diameter of the nucleus of the thyroid epithelium, μm	6.63(0.15)	5.37(0.12) <0.01 ¹⁾	6.28(0.14) >0.05 ²⁾ <0.01 ³⁾
Height of follicular epithelium, μm	13.85(0.32)	12.79(0.30) <0.05 ¹⁾	12.44(0.29) <0.05 ²⁾ >0.05 ³⁾
Nucleocytoplasmic index, RU	0.010(0.001)	0.014(0.001) <0.05 ¹⁾	0.014(0.001) <0.05 ²⁾ >0.05 ³⁾
Percent follicular epithelium volume, %	37.20 (1.86)	32.70(1.64) >0.05 ¹⁾	35.60 (1.78) >0.05 ²⁾ <0.05 ³⁾
Percent interfollicular epithelium volume, %	22.30(1.12)	25.10(1.26) >0.05 ¹⁾	24.40(1.22) >0.05 ²⁾ >0.05 ³⁾
Percent stroma volume, %	24.60 (1.23)	30.30(1.47) <0.05 ¹⁾	26.50(1.33) >0.05 ²⁾ >0.05 ³⁾
Percent vascular bed volume, %	15.70 (0.79)	11.60(0.64) <0.01 ¹⁾	13.40(0.67) >0.05 ²⁾ >0.05 ³⁾

Notes:

n - number of animals in the group;

\bar{x} - arithmetic mean;

s - standard deviation;

¹⁾ - statistical significance of differences between gr. 2 and gr. 1 by Dunn's criterion;

²⁾ - statistical significance of differences between gr. 3 and gr. 1 by Dunn's criterion;

³⁾ - statistical significance of differences between gr. 3 and gr. 2 by Dunn's criterion

Conclusions. Morphofunctional criteria of instability and changes in the microstructure of TG of offspring whose mothers were exposed to nicotine in postnatal life were determined. Primary apoptotic-destructive processes in the parenchyma, the formation of unstructured cell fields and microfollicles allow us to consider these offspring as a risk group for thyroid pathology in terms of additional exposure to such negative factors as "passive" smoking.

Prospects for further research. Prospects for further work on the problem are as follows: to create and experimentally investigate a new pharmacological composition capable to protect the functional systems of the fetus from the negative effects of tobacco-smoking products, both active and "passive".

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