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MORPHO-FUNCTIONAL STATE OF HUMAN FETUSES' ADRENAL CORTEX DURING INFLUENCE OF MATERNAL INFECTION

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Abstract

Aim of study. To reveal influence of maternal infection on morpho-functional state of fetuses' adrenal cortex.

Methods. The material was obtained during sectional studies, which were conducted on the basis of the "Kharkiv Regional Clinical Perinatal Center" from 2012 to 2016. The sample included fetuses with a gestation period of 32-42 weeks. The adrenal glands of fetuses were examined morphologically, using histological, morphometric and immunohistochemical methods.

Results. It was found that maternal infection leads to a decrease of morpho-functional activity of constant and fetal zones of the adrenal cortex, which was manifested by hypoplasia of zona glomeroulosa and fetal zone depletion; cytolysis and resorption of spongiocytes in zona fasciculata. In this case, increase in hormonal production in the permanent cortex is a manifestation of compensatory-adaptation reaction.

Key words. Fetus, maternal infection, hypoxia, adrenal glands

Introduction. One of the most important problems of pediatrics, obstetrics and gynecology is success of pregnancy and birth of healthy children. The success of pregnancy is determined by complex interaction of mother, placenta and fetus, development of fetus and its adaptation to changing environmental conditions [1, 2, 3].

Maternal factors (somatic diseases, pathological conditions during pregnancy, pathological labor) lead to increase number of infants with an extremely low body weight, affect degree of severe pathology development in early neonatal period, as well as lengths of adaptation period of newborn to extrauterine existence [4, 5].

According to WHO, over the past ten years, the perinatal mortality rate is gradually declining, mainly due to premature babies, namely thanks to the success of clinical and organizational tactics. At the same time, many authors note that with expressed attention of doctors to problem of miscarriage and preterm labor, the losses among full-term children have significantly increased [6].

Among causes of neonatal mortality in recent years, intrauterine infection of the fetus (IUI) occupies a leading place, causing from 11% to 45% of deaths rate [7]. The closest companion of mother infectious pathology is chronic intrauterine hypoxia (CIH). CIH in children can lead to various chronic diseases, deterioration in life quality and sometimes to disability and death [8].

In formation of adaptation processes to various environmental factors, an essential role belongs to endocrine system and in particular to adrenal gland, as one of its leading links [9].

However, despite the large occurrence, the question of pregnant women infection effect on the morphological characteristics of fetal organs, and in particular the adrenal glands, has not been studied. Information in literature is inconsistent and few. [10]

The aim of the study was to reveal the influence of maternal infection on the morphofunctional state of fetuses' adrenal cortex.

Materials and methods. Clinical material was obtained during sectional studies, which were carried out on the basis of "Kharkiv Regional Clinical Perinatal Center" in Ukraine from 2012 to 2016. The study included fetuses with a gestation period of 32-42 weeks.

Cases were allocated based on presence or absence of infectious pathology in mothers. The obtained sections were divided into 3 groups: the control group (the fetuses from mothers with physiological pregnancy), the comparison group (the fetuses from mothers whose pregnancy was complicated by chronic intrauterine hypoxia - CIH), the group of infections

(the fetuses from mothers whose pregnancy was complicated by infections, caused by various pathogens). Every group contained two subgroups – preterm fetuses and full-term fetuses.

From all the fetuses we extracted adrenals, fixed in 10% formalin, then poured into paraffin. A histological method was used: staining of sections with hematoxylin and eosin for observational microscopy. Micro-preparations were studied on the microscope "Olympus BX-41". Morphometric examination was carried out on computer images. We calculated relative volumes of main structural components, cell density of each zone, area of nuclei, cells and nuclear-cytoplasmic ratio using Photoshop CS5.

Immunohistochemical study was carried out on paraffin sections 5-6 μm thick by the indirect Coon's method according the modification of M. Brosman [11] (1979). We used antiserum to cortisol (Novocastra Laboratories Ltd, UK) for highlighting hormone-productive cells. Interleukin-producing cells were determined using monoclonal antibodies for interleukin-6 (IL-6) and tumor necrosis factor-α (TNF) Novocastra Laboratories Ltd. Collagens were typed with monoclonal antibodies for to Type I collagen (Novocastra Laboratories Ltd.) and Type III (IMTEK, Ltd, Russia). As fluorescent labels we used F (ab) - 2 fragments of rabbit antibodies against mouse immunoglobulins labeled with FITC (fluorescein isohiocyanate). The slides were studied in a fluorescent microscope "Axioskope 40" (Carl Zeiss, Germany). The optical density of immunofluorescence was measured by the method of Gubina-Vakulik GI et al. [12] and expressed in conventional units of luminescence (c.u.l.). The data was statistically processed using licensed application package Microsoft Exel. The methods of variational, alternative analysis were used. For significance calculations we used a nonparametric Mann-Whitney U-test and chi-square Pearson test. [13, 14]

Results and discussions

After conducting our own research and studying the literature data, we came to conclusion about negative impact of infectious mother pathology on fetuses' adrenal glands and its influence on the processes of morphogenesis with formation of endocrine pathology possibility in the future.

During macroscopically examination of all groups fetuses' adrenal glands, it is established that they had a round-triangular shape, covered with a connective tissue capsule. A cortical and medullar substance was determined on incision. Microscopically definitive cortex is already well contoured. The cortex cells form epithelial cords, intervals between them were filled with a loose connective tissue, in which blood capillaries and nerve fibers pass.

Zona glomeroulosa in all fetuses of the control group is formed by small irregularly shaped "glomeruli", which contain small cells with a light cytoplasm and a small dense dark nucleus. At full-term fetuses arcade structures were clearly visible. In preterm fetuses, arcade structures are only visible in places. Zona fasciculata occupied the middle part of the organ. Its cells were large in size, more cubic and prismatic. The zone is represented by spongiocytes mainly with light oval nuclei and eosinophilic cytoplasm. But there were small dark cells with a dense dark nuclei. The fetal zone dominated in the control group in all fetuses. It is represented by densely located cells with moderately bright nuclei in which nucleoli were seen, and a rich acidophilic cytoplasm. Its cells were more cubic. Immunohistochemical study of hormone production with antiserum to cortisol in the control group showed moderate expression of cortisol in the definitive cortex, which indicates the normal development of the adrenal glands and preparation for the birth stress. An investigation of type I and III collagens showed their moderate presence in the vessels' walls and in the organ capsule. In some places, small, weakly luminescent cells with expression of IL-6 and TNF-α are noted, which indicates the permeability of maternal antibodies to the adrenal gland of the fetus.

During microscopic study, it was found that in the comparison group (CIH), connective tissue capsule covering the adrenal glands is more loose and defibrated, which was not observed in the control group. In subgroup of full-term fetuses, the relative volume of zona glomerulosa had a certain tendency to increase ($p = 0.525$), although the cells density was significantly reduced ($p = 0.02$), i.e. the cells were more friable. At the same time, the nuclear-cytoplasmic ratio ($p = 0.021$) increased, which, possibly, indicates an increase in the functional capacity of cells in this zone. In the subgroup of preterm fetuses, on the contrary, there was a tendency to decrease of zona glomeroulosa ($p = 0.795$), while the cells density and their size (cell area $p = 0.013$) decreased reliably ($p = 0.02$). However, nuclear-cytoplasmic ratio increased, as well as in the subgroup of full-term fetuses ($p = 0.003$). (pic. 1)

In all fetuses of the comparison group, the cells of zona fasciculata were quite loosely contained among the loops of connective tissue. In most cases, foci of cytolysis and resorption of spongiocytes, which occupied up to 3.6% of the volume of zona fasciculata in full-term fetuses and up to 4.0% in preterm fetuses, were detected. In both subgroups, hyperplasia of zona fasciculata was observed (full-term fetuses $p = 0.001$, preterm ones $p = 1.4576E-06$), with an increase in cell density (full-term $p = 0.001$, preterm $p = 0.05$), the cells area of zona fasciculata is also significant increased (full-term $p = 0.0227$, preterm $p = 0.0005$). At the same time, the nuclei area only had a tendency to increase.

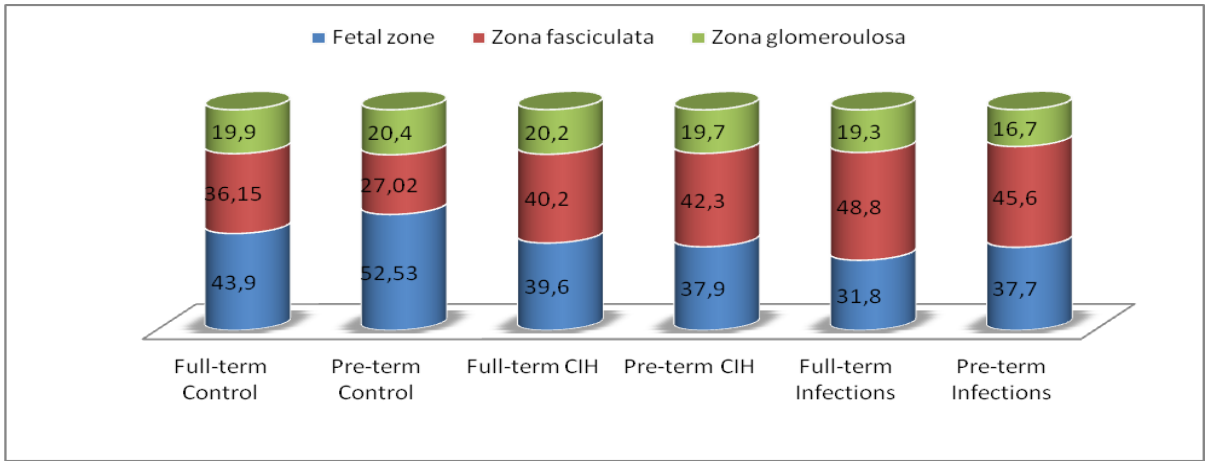
Microscopic examination of the fetal cortex revealed the presence of giant cells, which are characterized by high level of hormonal production. When compared with the control group, the cell density and the sizes of adrenocorticocytes were significantly decreased ($p = 0.01$ for both subgroups). Foci of cytolysis and cell resorption were noted, which are evidence of holocrine type of secretion of the adrenal cortex. These foci occupied up to 2.6% of the fetal zone.

In the group of infections in study of zona glomeroulosa, there was a clear tendency to reducing. In full-term fetuses, this was associated with a decrease in number and area of cells ($p = 0.004$). At the same time, a significant increase in the nuclear-cytoplasmic ratio was recorded ($p = 0.0024$). In preterm fetuses against the background of a significant reduction of zona glomeroulosa, the number of cells, their area and the area of their nuclei were increased. In these observations, there was also a significant increase in nuclear-cytoplasmic ratio ($p = 0.03$).

In zona fasciculata of both subgroups of the group of infections, a certain erasure of columnar structure was found in 32.4% of the observations. The incidence of this pathomorphological criterion was not significantly different in preterm and full-term fetuses (35.9% and 27.6%, respectively ($p = 0.324$)). At microscopic research in zona fasciculata huge cells were found out. In both subgroups, hyperplasia of zona fasciculata was observed (full-term $p = 0.003$, preterm $p = 1.8178E^{-08}$), with increasing cell density (in full-term $p = 0.002$, in preterm $p = 0.003$), the area of the cells was also significantly increased (full-term $p = 3.2565E^{-10}$, in preterm $p = 5.2657E^{-10}$), as well as the area of the nuclei (full-term $p = 0.048$, preterm $p = 0.002$), and in nuclear-cytoplasmic ratio (full-term $p = 0.0027$; preterm $p = 1{,}0275E^{-08}$). In most cases, foci of cytolysis and resorption of spongiocytes were detected. These zones occupied up to 5.1% of the volume of zona fasciculata in full-term infants and up to 5.3% of the volume in premature infants ($p = 0.013$ when compared with controls). In many observations, mitotic activity was detected.

The fetal zone was significantly and severely contracted, both in full-term fetuses ($p = 7.584E^{-06}$) and in preterm ones ($p = 0.0001$), but the number of cells in it is approximately equal to the comparison group, and the cells themselves are approximately equal, although have a tendency to decrease due to cytoplasm. In the fetal zone of the adrenal cortex, there were also foci consisting of denuclearized cells, which occupied up to 3.0% of the volume of fetal cortex. In premature fetuses in the fetal cortex, cytolysis and cell resorption sites were detected, which occupied up to 4.0% of the volume of the fetal zone.

Morphometric features of fetuses' adrenal gland cortex can be seen on pic.1



Pic. 1. Relative volumes of main structural components of adrenal cortex of fetuses from all groups

In the hormone production study, it was found that in all subgroups the processes of cortisol synthesis in the spongeocytes of zona fasciculata and fetal adrenocorticocytes were significantly increased. The only exception was in fetal zone in preterm fetuses from comparison group. In the comparison group: in full-term $p = 0.05$ in zona fasciculata, and $p = 0.0005$ in fetal zone; while in preterm $p = 0.05$ in zona fasciculata, $p = 0.208$ in the fetal zone. In the group of infections: in full-term fetuses $p = 6{,}3743E^{-22}$ in zona fasciculata, and $p = 4{,}3397E^{-09}$ in fetal zone; in preterm $p = 1.2729E^{-06}$ in zona fasciculata, $p = 0.0006$ in fetal zone. The received data, in our opinion, testify to a high functional tension of compensatory processes in the adrenal cortex in fetuses from infected mothers. Decrease of hormone production in fetal zone of the preterm fetuses in comparison group testifies to disruption of the adaptation processes.

In the comparison group, correlation analysis revealed the presence of a reliable positive moderate relationship between the intensity of luminescence of cortisol in zona fasciculata and the nuclear-cytoplasmic ratio $r = +0.44$ ($p < 0.05$). A weak positive dependence was also recorded between the intensity of luminescence of cortisol in the fetal zone and nuclear-cytoplasmic ratio $r = +0.37$ ($p < 0.05$). In preterm fetuses, there was no correlation found between the intensity of luminescence of cortisol in zona fasciculata and fetal zone and nuclear-cytoplasmic ratio, which may indicate a functional depletion of the spongiocytes (for zona fasciculata, $r = +0.07$ ($p < 0.15$) and for the fetal zone $r = +0.02$ ($p < 0.11$)).

In the group of infections, the correlation analysis we conducted revealed a positive moderate relationship between the cortisol luminescence in zona fasciculata and the nuclear-

cytoplasmic ratio r = +0.57 (p <0.05), and a moderate positive relationship between cortisol luminescence intensity in the fetal zone and nuclear-cytoplasmic ratio r = +0.43 (p <0.05) in full-term fetuses. In preterm fetuses, similar data were obtained (r = + 0.58, p <0.05 for zona fasciculata and r = +0.41 (p <0.05) for fetal zone.) Based on the obtained data, it can be concluded that with an increase in nucleus size is an increase in hormone production cells.

In immunohistochemical study of fetuses adrenal cortex, the thickening of connective tissue septa was found in the comparison group with a significant increase in the expression of type I collagen, both in full-term fetuses (p = 5,8555E-19) and in preterm fetuses (p = 0.006). In fetuses from infected mothers, more active expression was observed with type III collagen. In both subgroups, significant differences were observed (in full-term p = 0.008, in preterm p = 0.0001). In our opinion, these data indicate that the fetuses from infected mothers are always infected with chronic hypoxia, which according to the world literature promotes collagen formation. In addition, according to different authors, cytokine activity in the fetus can also help to increase the formation of "young" collagen. It is also known that cytokines regulate the activity of hormonal hypothalamus-pituitary-adrenal glands axis: for example, Interleukin-1, acting on the hypothalamus, enhances the synthesis of corticoliberin, which, in turn, increases the production of adrenocorticotropic hormone. [9, 13, 14]

In our study, in both subgroups of fetuses from infected mothers, an increase in the number of cells of interleukin-6-producers, as well as the tumor necrosis factor-α producer cells, was detected. In full-term fetuses - IL-6 producers - 10.4 ± 0.5 specimens in the x400 field of view; TNF α-producers - 8.3 ± 0.2 specimens. In preterm fetuses: IL-6-producers - 7.4 ± 0.03 specimens in the field of view x400; TNF α-producers - 11,3 ± 0,02 cells.

The correlation analysis in both subgroups revealed a positive moderate relationship between the number of cells of TNFα producers and type III collagen (in full-term fetuses: r = +0.45 (p <0.05), in preterm: r = +0.42 (p <0.05)), and a positive relationship was observed between the number of interleukin-6-producing cells and type III collagen (in full-term fetuses r = +0.49 (p <0.05), in preterm r = + 0.43 (p <0.05)), which was not observed in other study groups.

Probably, the accelerated maturation of the fetal adrenal glands, under the influence of hypoxic stress and maternal infection, can reverse the hypothalamic-pituitary-adrenal axis in reverse order, which in turn stimulates the immune response. In addition, from the data of our study, we can conclude that cytokines stimulate the production of a "young" type of collagen.

Conclusions:

1. In all studied groups, unidirectional changes were observed: hypoplasia of zona glomeroulosa and fetal zone depletion; cytolysis and resorption of spongiocytes in zona fasciculata. At the same time, in the group of infections, the changes were much bigger, which in our opinion is indicative not of a simple summation of symptoms, but of the induction of one pathological state by another.

2. A positive correlation was found between the intensity of luminescence of cortisol and nuclear-cytoplasmic ratio, both in the fetal zone and in zona fasciculata. In our opinion, as the nucleus increases, the functional activity of cell increases.

3. The increase in the number of cells IL-6 and TNF-α producers were observed in the fetuses from infected mothers, and in both subgroups there was a positive moderate relationship between the number of TNF-α producer cells and the number of IL-6 producers and collagen III type. In our opinion, these data indicate that in the fetuses from infected mothers, not only the hypoxic condition promotes collagen formation, but also the increased activity of cytokines.

4. The data obtained by comparison of full-term and premature fetuses testify to the immaturity of compensatory-adaptive mechanisms in the latter, which is manifested by severe depletion of the fetal cortex and accelerated maturation of the adrenal gland in response to stress.

It should be noted that the above-mentioned morpho-functional features of various areas of the adrenal cortex are reflection of overall reaction of child's body, and its adrenal glands, the hormones of which play a dominant role in adaptation processes, to the conditions of fetus that change in comparison with physiological pregnancy, and as a consequence, there is an acceleration of maturation and differentiation of the adrenal gland.

References

1. Andreyev A.V., Gubina-Vakulik G.I. [Perinatal'naya gipoksiya kak prichina patologicheskikh izmeneniy v nadpochechnikakh plodov i novorozhdennykh] - Mezhdunarodnyy meditsinskiy zhurnal, 2013, № 3

2. V.V. Vlasyuk Morfologicheskaya diagnostika vnutriutrobnykh infektsiy. Uchebnoye posobiye. SPb, 2010 — 47 s

3. H. M. Beere and D. R. Green, “Stress management—heat shock protein-70 and the regulation of apoptosis,” Trends in Cell Biology, vol. 11, no. 1, pp. 6–10, 2001.

4. S. N. Mateev, R. Mouser, D. A. Young, R. P. Mecham, and L. G. Moore, “Chronic hypoxia augments uterine artery distensibility and alters the circumferential wall stress-strain relationship during pregnancy,” Journal of Applied Physiology, vol. 100, no. 6, pp. 1842–1850, 2006.

5. V. Ananth and A. M. Vintzileos, “Epidemiology of preterm birth and its clinical subtypes,” Journal of Maternal-Fetal and Neonatal Medicine, vol. 19, no. 12, pp. 773–782, 2006.

6. Pretorius C. The relationship between periodontal disease, bacterial vaginosis, and preterm birth text. / C. Pretorius, A. Jagatt, R. F. Lamont // J. Perinat. Med. -2007. Vol. 35, № 2. - P. 93 - 99.

7. Mekhdiyeva YU.D., Murzabayev KH.KH., Fayzullina R.M. «Vliyaniye vnutriutrobnogo infitsirovaniya ploda na razvitiye patologii timusa i kory nadpochechnikov». «Uspekhi sovremennogo yestestvoznaniya» 2009 № 2

8. Jakobovits A. Intrauterine infections // Orv. Hetil. 2006. - №2. - P.287-290

9. Vladimirova, N.YU. Sovremennyye aspekty nevynashivaniya i nedonashivaniya beremennosti (uchebnoye posobiye) Tekst. / N.YU. Vladimirova, G.V. Chizhova. Khabarovsk: Izd. tsentr IGZHSZ. - 2005. — 21s

10. Samoylova A.V. Vnutriutrobnaya infektsiya v strukture zabolevayemosti i smertnosti novorozhdennykh Tekst. / A.V. Samoylova, L.G. Nog-teva // Mat-ly II region, nauchnogo foruma «Mat' i ditya», Sochi. 2008. -S.252-253.C.

11. Brosman M. Immunofluorestsentnoye issledovaniye formalin-parafinovogo materiala. // Cs. Patol.- 1979.- 15.- 4.- s. 215-220oC.

12. Gubina-Vakulik G.I., Sorokina I.V., Markovskiy V.D., Kikhtenko O.V., Kupriyanova L.S., Sidorenko R.V., izobretateli; KHNMU «Metod kolichestvennogo opredeleniya antigena v biologicheskikh tkanyakh» / Ukrainskiy patent na poleznuyu model' UA 46489 G01N33 / 00. №u 200906730; 25.12.2009.Bul.№4

13. Zaytsev V.M., Liflyandskiy V.G., Marinlin V.I. [Prikladnaya meditsinskaya statistika] Sankt-Peterburg: Foliant, 2003. 432p.

14. Lapach S.N., Chubenko A.V., Babich P.N. Statisticheskiye metody v mediko-biologicheskom issledovanii s ispol'zovaniyem Exel \ - K.: MORION, 2001. - 408p