



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating 2017: 5.03  
TPI 2017; 6(4): 107-110  
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www.thepharmajournal.com  
Received: 19-02-2017  
Accepted: 20-03-2017

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## Effect of mother's infection with staphylococcus aureus on Morpho-functional state of offspring's adrenal cortex (Experimental research)

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### Abstract

Experimentally we found that in mother's infected with *S. aureus*, the fetuses adrenal glands have peculiar morphological features, that show decrease in morphological and functional activity of the definitive zone, but increased hormone productivity in the fetal zone of the adrenal cortex which is a manifestation of the compensatory-adaptive process in response to intrauterine infection.

**Keywords:** Adrenals, rat, fetus, intrauterine infection

### Introduction

Intrauterine infection of the fetus is one of the most important problems of modern obstetrics. According to WHO, in the world each year about 5 million fetuses and newborns die. Among the main causes of their deaths - infections, extreme morphological and functional immaturity, birth asphyxia, and others [1]. In recent years there has been an upward trend of infectious diseases associated with opportunistic microflora. This connection, changed the structure of infectious diseases of pregnant women, fetuses and newborns. Today among the microorganisms that penetrate into the uterine cavity and lower parts of the reproductive system, are prevalent opportunistic bacteria. Microbial imbalance of the vaginal environment can also be considered as the main cause of complications of pregnancy and intrauterine infection. [2] The most common disorder of the vaginal microflora, whose frequency in pregnant women is 10-20%, is dysbiosis, which is characterized by a sharp decrease of the representatives of the normal microflora and its replacement by a mixed flora consisting of anaerobic opportunistic bacteria. [3] The greatest danger during pregnancy is Staphylococcus aureus. Staphylococcus aureus is still one of the four most common causes of hospital-acquired infections. [4]

Thus, taking into account the role of opportunistic microflora in the development of intrauterine infection and contradictory literature data, the study of changes in morphology and function of the adrenal glands during *S. aureus* infection of the mother is necessary for understanding the pathogenesis.

**Goal:** To identify experimentally the effect of maternal infection caused by *S. aureus* on the state of the adrenal cortex of rat's offspring.

**Materials and methods:** For the experiment we formed 3 groups of animals - WAG female rats: control group - intact animals, which were kept under physiological conditions with normal pregnancy; comparison group or a group with chronic intrauterine hypoxia (CIH) - a group of rats that were exposed to hypoxia; and main group - rats have been infected with *S. aureus*.

Intrauterine infection of fetus and chronic intrauterine hypoxia should be considered as conditions that require a lot of adaptive changes, in which one of the most important roles is played by the adrenal gland. So we decided to conduct two experiments, the results of which will help us to distinguish between "clean" CIH from CIH that accompanies the infection.

In the first experiment we modeled CIH. For twenty-one days (the pregnancy period) female rats were subjected to every day alpine hypoxia. In the later stages of gestation the females were taken out of the experiment. Fetuses were removed for study. For CIH simulation we used a sealed pressure chamber, from which air was pumped out, thereby creating conditions

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for a sharp decrease in atmospheric pressure. Rats each day at the same time for 20 minutes were placed in conditions that correspond to a height of 7500 m (pressure 287 mm. Hg. V.) [5]

Second experiment: infected rats. It was conducted in two stages. In the first stage we found infecting dose of subacute, sustained, infectious and inflammatory processes in the abdominal cavity of female rats (as an infectious agent we used reference strain *Staphylococcus aureus* (ATCC 25923)). In the second stage we placed males with female rats, rats became pregnant, from pregnant females we collected the fetuses. [6] Removing animals from the experiment was conducted according to conditions specified in the methodical recommendations of Ministry of Health of Ukraine, and the ethical principles of animal experiments in accordance with the provisions of the "European Convention for the Protection of Vertebrate, which was used for experimental and other purposes."

In fetuses of all groups both adrenal glands were removed to get the average results. Organs went through standard histological wiring: fixation in 10% formalin, and then fill in paraffin. Preparations were stained with hematoxylin and eosin and pikrofuksin by Van Gison method for microscopy review. We used microscope «Olympus BX-41» to study slides. Corticosterone-producing activity in the adrenal cortex of experimental animals was studied by indirect method Coons by method Brosman (1979) [7] using an antiserum to cortisol. As fluorescent labels used F (ab) -2 - fragments of rabbit anti-mouse immunoglobulins labeled with FITC. The preparations were investigated in luminescent microscope «Axioskop 40» (Carl Zeiss, NGF). Immunofluorescent optical intensity density was measured by the method of Gubina Vakulik G.I. and coauthors [8] and it was expressed in arbitrary luminescence units (arb.lum.u.)

The morphometric study was carried out on computer images: we calculated relative volumes of the main structural components of cell, counted density of cells for each zone, the area of nuclei and cytoplasm of cells in the adrenal glands, the study was carried out with the help of Photoshop CS5 software. Statistical data was processed by methods of mathematical statistics using variations and alternative assays using the package of Microsoft applications "Exel - 5.0". [9, 10]

## Results

In all groups, the adrenal glands had rounded-triangular shape and were covered with connective tissue capsule in which two layers were determined microscopically - looser inner and outer denser. Microscopic examination revealed that cells of the cortex formed oriented perpendicular strands, the gaps between them were filled with loose connective tissue in which blood vessels and nerves pass.

In the study of the control group, the zona glomerulosa consisted of small endocrine cells that form rounded clusters or "glomerulus". Zona glomerulosa was well differentiated from the zona fasciculata through a layer of dark cambium cells. Cells of zona glomerulosa had bright eosinophilic cytoplasm and a small nucleus. Zona fasciculata of adrenal cortex occupied the middle of the bands and in control group of fetuses was narrow. Cells had pale eosinophilic cytoplasm.

In rats of control group fetal zone was the biggest and showed tightly spaced endocrinocytes with light nuclei.

In rats, the comparison group of chronic intrauterine hypoxia histological structure of the adrenal was different from that of the control group. Zona glomerulosa of the adrenal cortex was increased, due to loose arrangement of cells, formed by small dark cells. Reduction in cell density was detected in this zone, there was a significant increase in the cell area due to the cytoplasm, as evidenced by almost equal core area. (Table 1)

The width of zona fasciculata in the comparison group as a result of sclerotic lesions was significantly increased compared with the control group. The area of lesions revealed cytolysis and resorption of cells. The zone is represented by rounded light cells with clear cytoplasm. The cell density in the field of view X400 is equal to that of the control group, reduction in area of cell occurs due to reducing volume of cytoplasm, as evidenced by an increase in the core area. Immunohistochemical study with antiserum to cortisol found on active immunofluorescence in zona fasciculata: (Table 2)

Fetal cortex is reduced in width, with a tendency to decrease in average cell area, but there is also a tendency to increase in core area. In this zone are found endocrinocytes in cubic form with a light cytoplasm. In fetal cortex immunofluorescence of cortisol is reduced in comparison to the control group (Table 3).

In rats of the main group histological picture differed from the control group and the comparison group. In the study of zona glomerulosa it is represented by small dark cells with a small dense core. Glomerular zone of the cortex was significantly reduced considerably, there is a tendency to a decrease in cell density in sight X400 also observed a significant reduction in the cell area, the core area in this case has remained virtually unchanged. (Table 1)

**Table 1**

Parameters	Control	CIH	S.aureus
Zona glomerulosa:			
Width in absolute figures, mkm	32,68±0,99	25,04±0,50 <sup>1</sup>	19,30±0,4 <sup>1</sup>
in percents, %	16,04±0,24	17,55±0,46 <sup>#</sup>	10,32±0,33 <sup>1</sup>
Number of cells, in field of view	113,10±3,76	76,50±5,39	69,2±0,78
Cell area, mkm <sup>2</sup>	30,59±0,34	35,28±0,53 <sup>#</sup>	25,27±0,14 <sup>1</sup>
Nucleus area, mkm <sup>2</sup>	11,52±0,16	11,79±0,21	11,56±0,07

**Note:** \* -  $p \leq 0,05$ , # -  $p \leq 0,01$ , 1 -  $p \leq 0,001$

Zona fasciculata contained cells with clear cytoplasm and light nucleus. There were dual-core cells. In zona fasciculata there were highly visible lesions of resorption and cytolysis of cells and the formation of voids. We found small eosinophilic granules in the cytoplasm. In some cells karyorhexis and karyolysis were seen. The width of the zona fasciculata increased as compared with the comparison group, there is a significant increase in the number of cells in the visual field, the cell nucleus area and thus significantly reduced. Immunohistochemical study revealed equal control immunofluorescence of cortisol in zona fasciculata. (Table. 2)

Table 2

Parameters	Control	CIH	S.aureus
Zona fasciculata:			
Width in absolute figures, mkm	54,61±1,20	57,94±1,89	84,36±2,95 <sup>1</sup>
in percents, %	26,85±0,42	40,45±0,95 <sup>#</sup>	44,76±1,1 <sup>#</sup>
Number of cells, in field of view	218,60±4,10	216,00±2,52	232,5±5,23 <sup>*</sup>
Cell area, mkm <sup>2</sup>	69,87±0,62	62,74±0,53 <sup>*</sup>	56,82±0,2 <sup>1</sup>
Nucleus area, mkm <sup>2</sup>	20,87±0,14	23,46±0,29 <sup>*</sup>	18,48±0,07 <sup>1</sup>
Immunofluorescence of corticosteron, (arb.lum.u.)	0,72±0,02	0,92±0,02 <sup>*</sup>	0,72±0,01 <sup>#</sup>

Note: \* -  $p \leq 0,05$ , # -  $p \leq 0,01$ , 1 -  $p \leq 0,001$

The fetal cortex had loosely arranged light endocrinocytes with light nucleus. The width of the fetal zone significantly increased, but significantly decreased the cell density, average

cell area and the core area also decreased. Determined immunofluorescence of cortisol in the fetal cortex was moderate (Table 3).

Table 3

Parameters	Control	CIH	S.aureus
Fetal zone:			
Width in absolute figures, mkm	116,23±2,60	60,00±0,88 <sup>1</sup>	84,92±3,48 <sup>1</sup>
in percents, %	57,11±0,52	42,00±0,62 <sup>#</sup>	44,91±1,03 <sup>#</sup>
Number of cells, in field of view	131,20±1,24	139,10±5,58	103,57±1,4 <sup>1</sup>
Cell area, mkm <sup>2</sup>	83,11±1,07	71,98±0,13 <sup>#</sup>	65,07±0,08 <sup>1</sup>
Nucleus area, mkm <sup>2</sup>	23,37±0,31	22,04±0,12 <sup>#</sup>	18,68±0,08 <sup>1</sup>
Immunofluorescence of corticosteron, (arb.lum.u.)	0,79±0,03	0,73±0,04	0,81 ± 0,02

Note: \* -  $p \leq 0,05$ , # -  $p \leq 0,01$ , 1 -  $p \leq 0,001$

## Discussions

It is known from the literature that in genotype complete structure of the immune system in conditions of prolonged ongoing antigenic effects, the leading role belongs to reactive changes in the hypothalamic-pituitary-adrenal system, which leads to an increase in blood corticosteroids, thereby providing a stress effect on the formation of organs and systems of the fetus [2-4].

With increased functional stress in endocrine organs of the fetus pathological processes occur: hypertrophy and hyperplasia of the secretory cells, morphological reorganization of the endocrine glands. In chronic intrauterine hypoxia results in the state of functional exhaustion, decompensation of endocrine organs of the fetus, degeneration and destruction of secretory cells. These processes are not so much a consequence of metabolic disorders, but as a manifestation of organism to move through emergency hyperstimulation of secretion by regulatory systems [4].

Thus, the morphological changes in the fetus show the severity and length of the pathological intrauterine process. Reaction to severe stress influences characterized by almost simultaneous, but different in the degree of involvement of all morphological and functional adrenal zones into a single adaptive response [11].

The changes revealed by us, namely severe hypoplasia of the zona glomerulosa and hyperplasia of the zona fasciculata with signs of exhaustion of the functional activity of cytolysis and resorption of cells and reduce of hormone production, as well as increasing the functional activity of the fetal zone can be considered as a manifestation of compensatory-adaptive process in response to intrauterine infection.

## Conclusions

1. When a mother is infected with *Staphylococcus aureus* in the definitive zone, the adrenal glands cortex of the fetus develops changes, manifested as severe hypoplasia and hyperplasia of the zona glomerulosa and zona fasciculata.

Zona fasciculata shows pronounced signs of exhaustion of functional activity as cytolysis and resorption of cells and decrease of hormone production.

- In the fetal zone of the adrenal glands, the cortex of the fetus developd's changes, manifested by increased functional activity of the fetal zone.
- These data support the adaptive reactions of the adrenal gland in response to antigen stimulation, in our study it appeared in maternal infection with *S.aureus*.

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