P.A. Kobeza

Dnipro State Medical University
Dnipro, Ukraine

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QUANTITATIVE AND QUALITATIVE ANALYSIS COMPONENTS THE MYOCARDIUM OF RATS IN HYPOXIA

Kobeza P.A. ✉ **Quantitative and qualitative analysis components the myocardium of rats in hypoxia.**
Dnipro State Medical University, Dnipro, Ukraine.
ABSTRACT. Background. Various types of hypoxia occur during fetal development and early neonatal phases, influenced by factors that complicate pregnancy. Given the frequency of cardiovascular issues, understanding the impact of antenatal hypoxia on newborn hearts and exploring corrective measures is crucial in medical research and practice. **Objective.** The study focuses on analyzing the quantitative and qualitative composition of contractile cardiomyocytes as the primary cell population in elements of the myocardial contractile apparatus. It involves examining the proliferative variability of components of the myocardial contractile apparatus under conditions of modeled hypoxia of varying types. Additionally, it includes a quantitative and qualitative analysis of the cellular composition of myocardial elements in adult rats and their offspring subjected to various types of hypoxia during prenatal development. **Methods**. The study was conducted on sexually mature female Wistar rats (4-5.5 months) and their offspring – newborn rats (14, 16, 20 Prenatal and 1 day Postnatal). **The Results**. Under the influence of chronic hypoxia on the 14th day of prenatal development, different areas of the atrial myocardium demonstrated significantly different thickness values. The values of this indicator in the second experimental group did not significantly differ from the control group values at this term. The effect on the 16th day of prenatal development affected the proliferative activity. Following exposure to chronic hypoxia, this measure in newborn rats significantly increased in RV, and in the LV compared to the previous term. On the 16th day of prenatal development, the thickness values of the atrial and auricular myocardium in animals from the first experimental group did not significantly differ from the values of the previous term and the control group. The myocardial thickness on the 20th day after the influence did not significantly differ compared to the 16th day of prenatal development and compared to the norm. The proliferative activity of atrial cardiomyocytes in animals from the first experimental group gradually decreased until the first day of postnatal development, which was reflected in the reduction in the number of Ki-67 positive cells compared to the 16th day. **Conclusion.** Under conditions of hypoxia and in normal conditions from the 14th day of prenatal development to the 1st day of postnatal development, the myocardial thickness demonstrates an inverse correlation with the proliferation index of atrial cardiomyocytes; under normal conditions. Hypoxia does not significantly affect the processes of proliferation of atrial cardiomyocytes.
Key words: tissue hypoxia, cardiomyocyte, contractile apparatus, ultrastructure, myofibrils.

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Kobeza P.A. 0000-0003-1113-4007
✉ **kobeza.pavel@gmail.com**

Background

Perinatal hypoxia has a significant impact on the neonatal myocardium and is associated with prevalent cardiovascular diseases during childhood. Various types of hypoxia occur during fetal development and early neonatal phases, influenced by factors that complicate pregnancy [1]. Urban stressors, ranging from emotional burdens to industrial toxins, affect pregnant women, often leading to fetal hypoxia. This hypoxia negatively affects fetal organs, causing developmental delays, imbalances in biogenic amines, and damage to the nervous system and heart in affected children. Given the frequency of cardiovascular issues, understanding the impact of antenatal hypoxia on newborn hearts and exploring corrective measures is crucial in medical research and practice [2]. Extended fetal pathology significantly contributes to the increased incidence of neonatal and childhood pathologies. Newborns exhibit hypoxic damage to the cardiovascular system at rates of 40-60%. Changes in myocardial energy metabolism lead to a rapid decline in contractile function, driven by intricate metabolic changes postcerebral pathology and exacerbated by disrupted cardiovascular autonomic regulation due to excessive sympathetic activation [3]. The impact of ante-

natal hypoxia on newborns depends on its nature, individual tolerance, and intrauterine development phases. It exacerbates congenital heart defects, initiating early heart failure and rhythm disturbances. Studies on sheep fetuses with intrauterine hypoxia revealed reduced heart rate linked to lowered blood oxygen saturation and arterial pressure, as evidenced by morphological studies indicating apoptosis and dystrophy in the heart's conducting system, correlating with the severity of detected arrhythmias [4].

Prolonged hypoxic heart damage may lead to focal cardiomyodystrophy, culminating in cardiomyosclerosis. Metabolic cardiomyopathies often stem from antenatal hypoxia, encompassing myocardial diseases with unclear origins that result in partial loss of myocardial contractile protein properties, hindering efficient cardiac muscle contraction [5]. Fetal responses to hypoxia include bradycardia, which could potentially cause circulatory hypoxia. The size of the fetus's heart mirrors its circulation. However, neither hypoxia nor hypercapnia triggers compensatory increases in uterine blood flow, adversely affecting the child's systemic circulation and worsening heart failure symptoms [6]. Post-hypoxic processes during labor vary, affecting different levels of cardiovascular damage: neonatal pulmonary hypertension, fetal communication occlusion, chamber dilation with myocardial dysfunction, ischemia, and disruptions in heart rhythm and conduction. Prolonged hypoxia burdens the heart, inducing vasoconstriction in both circulatory systems due to catecholamine release and hypercapnia [7]. Elevated blood flow to the heart raises right ventricular pressure, potentially matching systemic arterial pressure. Inadequate myocardial blood flow fails to sufficiently oxygenate cardiomyocytes, leading to inevitable myocardial ischemia. Residual effects, like moderate pulmonary hypertension and decreased myocardial contractility peaking from days 3-7, persist until around age three in one-third of newborns who underwent perinatal hypoxia [8]. An increase in hypoxia-induced factor HIF-1 significantly impacts gene transcription during embryonic development [9]. The ontogenetic changes in embryonic development rely on specific gene expression patterns governing crucial epigenetic mechanisms—such as DNA methylation, chromatin remodeling, histone modification, and post-translational modification. Hypoxia stabilizes HIF-1α and increases free radicals in the fetal myocardium, activating epigenetic modifiers. These modifiers heighten methylation at transcription factor binding sites and deacetylation of histone residues, obstructing the transcription of cardioprotective genes, ultimately reducing inherent cardioprotection in the long term [10]. This study delves into the intricate aspects of hypoxic heart damage, exploring its consequences, outcomes, and molecular mechanisms associated with perinatal and fetal hypoxia.

The objective to determine quantitative changes in the proliferation and growth processes of cardiomyocytes in the atria of rats under the influence of acute and chronic intrauterine hypoxia during prenatal development.

Methods

The study focuses on analyzing the quantitative and qualitative composition of contractile cardiomyocytes as the primary cell population in elements of the myocardial contractile apparatus. It involves examining the proliferative variability of components of the myocardial contractile apparatus under conditions of modeled hypoxia of varying types. Additionally, it includes a quantitative and qualitative analysis of the cellular composition of myocardial elements in adult rats and their offspring subjected to various types of hypoxia during prenatal development. Experimental design, models used, dosage, and administration frequency. The study was conducted on sexually mature female Wistar rats (4-5.5 months old, body weight – 200-230g) and their offspring – newborn rats (14, 16, 20 Prenatal and 1 day Postnatal, body weight – 4.20-7.62g). To induce pregnancy, virgin females were mated with sexually mature males at a ratio of two males to four females. Pregnancy in rats was confirmed by the presence of spermatozoa in the morning vaginal smear (1st day of pregnancy). For the research purposes, the experiment was divided into groups. The first group aimed to investigate the morphofunctional features of the myocardium under conditions simulating histotoxic hypoxia. The second group served as the control, consisting of intact animals. Another group received intraperitoneal injections of physiological saline as a counterpart to the isolated administration of sodium nitrite.

The study was conducted on white non-pedigreed female rats and their offspring. A series of embryos at the 14th, 16th, and 20th days of prenatal ontogenesis and the hearts of newborn rats were used as the material. Animal maintenance and experiments were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [11], "General Ethical Principles of Experiments on Animals" adopted by the Fifth National Bioethics Congress. The age of the obtained embryos was determined based on a combination of external characteristics considering the gestation day according to the tables of normal embryonic development [12-14].

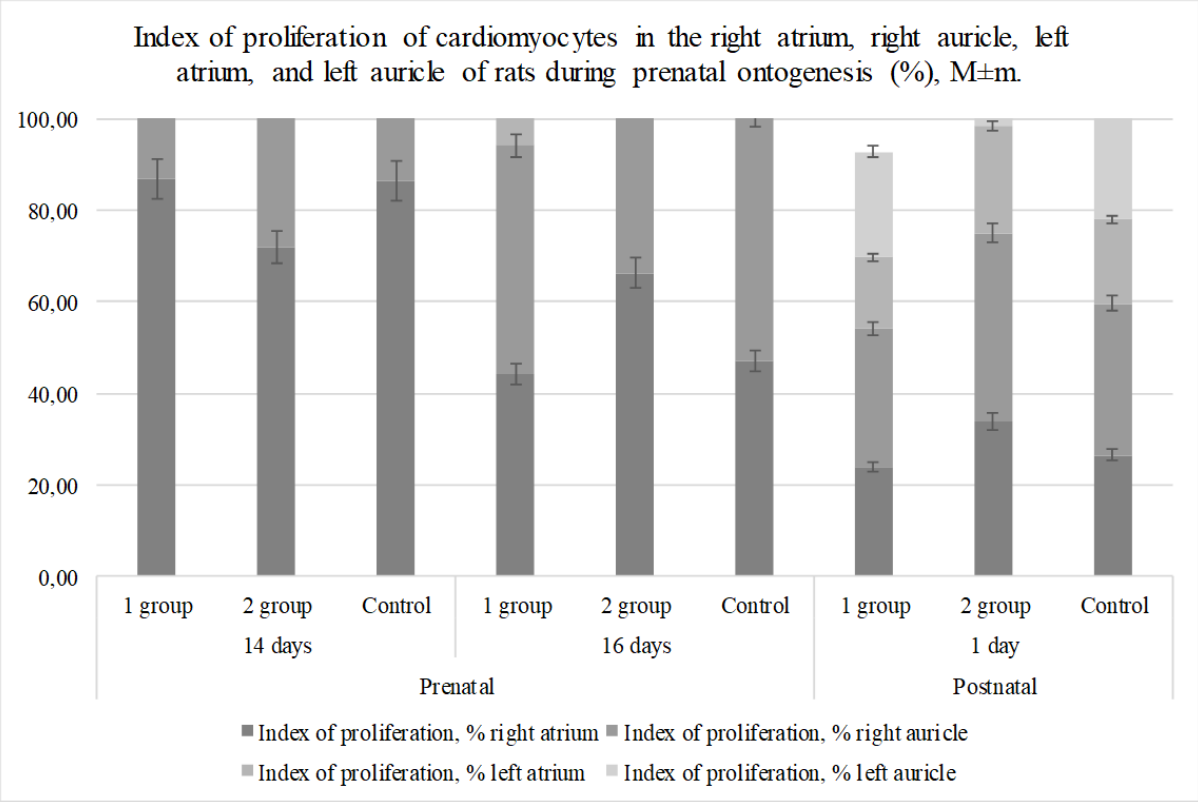
According to the research objective, the animals were divided into three groups: animals of the first experimental group subjected to acute hypoxia, animals of the second experimental group subjected to chronic hypoxia, and animals of the control group kept under standard conditions in the vivarium. Hypoxia was induced using a standard methodology in pregnant females by subcutaneously administering sodium nitrite in doses causing moderate hypoxia: a single dose of 5 mg/100 g body weight on the 13th

day of pregnancy for modeling acute prenatal hypoxia and doses of 5 mg/100 g body weight from the 10th to the 21st day of pregnancy for modeling chronic prenatal hypoxia.

Control animals were subcutaneously administered 1 ml of 0.9% physiological saline solution of sodium chloride. The embryonic material of the experimental animals was obtained in laboratory conditions following recommendations and commonly accepted methods for selecting laboratory material from experimental animals [15]. The material was fixed in a buffered formalin solution, dehydrated in increasing concentrations of alcohol, permeated with chloroform, and embedded in paraffin. Sections of 5 µm thickness were stained with hematoxylin and eosin. To establish the proliferative activity of cardiomyocytes, monoclonal antibodies Ki-67 were utilized. Immunohistochemical reactions were performed using the LSAB visualization system. Stained histological sections were examined under a Carl Zeiss Primo Star light microscope at magnifications of x200 and x400. Digital images captured with a Sigeta MCmos 3100 3.1MP camera were subsequently contrasted and analyzed using the ImageJ program (National Institutes of Health). Throughout the study, a complex of morphometric methods and standard biometric analysis was employed.

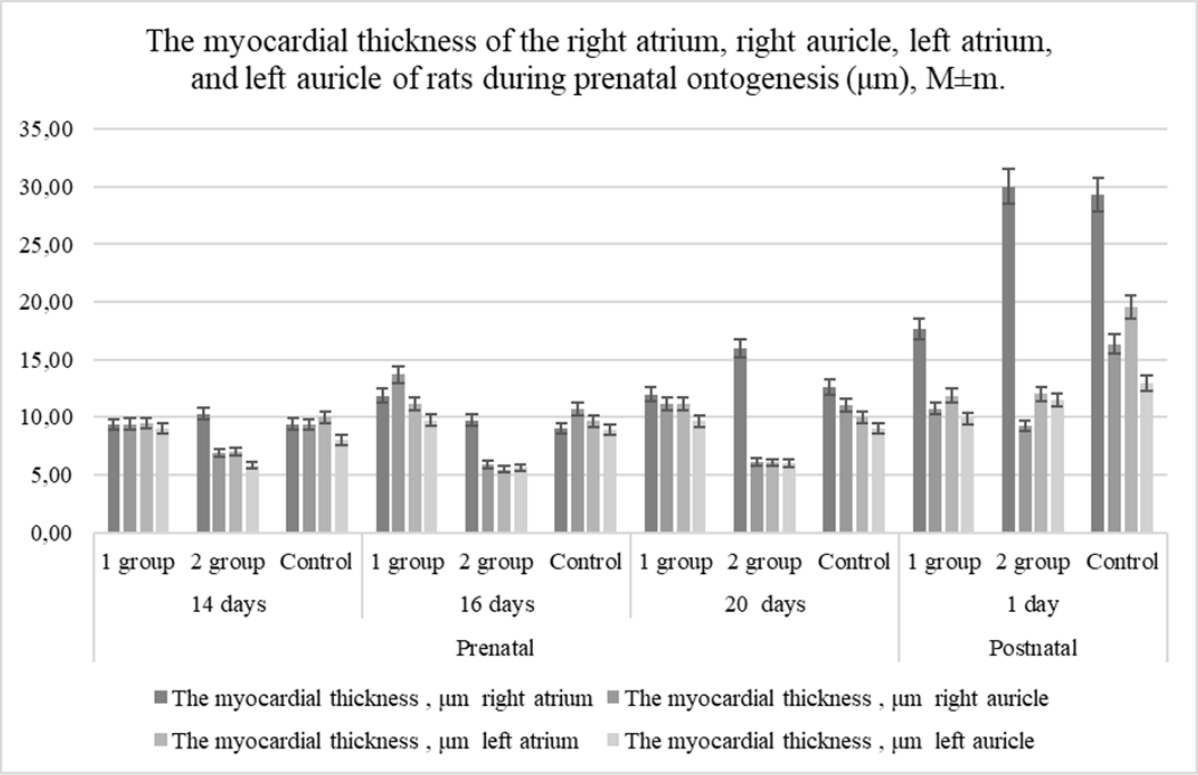
Results and Discussion

The quantitative analysis of morphological changes in the atrial myocardium was carried out in the areas of the atria and auricles separately for the right and left chambers. On the 14th day, against the backdrop of undeveloped auricles, the lateral zones of the right atrium (RA) and left atrium (LA) were examined [16]. Cardiomyocytes (CMCs) in the atria of rats displayed small sizes with round nuclei and high proliferative activity [17]. The proliferation index of atrial CMCs in the second experimental group did not significantly differ across various atrial areas compared to the control group at that time point [18-21]. The values of this indicator also did not significantly differ from the control group values at this term [22, 23].



Under the influence of chronic hypoxia (CH) on the 14th day of prenatal development, different areas of the atrial myocardium demonstrated significantly different thickness values: RA - 10.305 ± 0.517 µm, lateral zone of RA - 6.86 ± 0.63 µm, LA - 6.97 ± 0.12 µm, lateral zone of LA - 5.81 ± 0.58 µm. The values of this indicator in the second experimental group did not significantly differ from the control group values at this term. The effect of CH on the 16th day of prenatal development affected the proliferative activity of CMCs: the proliferation index values of atrial CMCs in the second experimental group were significantly higher by 44.7% ($p<0.05$) in RA, by 54.7% ($p<0.05$) in the right auricle (RA), by 34.4% ($p<0.05$) in LA, and by 57.8% ($p<0.05$) in the left auricle (LA) compared to the norm, but did not significantly differ compared to the previous term. Under the influence of CH on the 16th day of prenatal development, the myocardial thickness values did not significantly differ from the

corresponding values at the previous term, but were lower by 41.4% ($p<0.05$) in RA, by 37.6% ($p<0.05$) in LA, and by 41.1% ($p<0.05$) in LA compared to the norm. The myocardial thickness values in the second experimental group on the 20th day of prenatal development were lower by 22.6% in RA, by 45.7% ($p<0.05$) in RA, by 41.4% ($p<0.05$) in LA, and by 39.4% ($p<0.05$) in LA compared to the norm, but did not significantly differ from the previous term.



The thickness of the myocardium in animals from the second experimental group reaches its maximum value at the 10th moment of birth in the right atrium - 31.04±5.11 μm, significantly higher by 117.9% compared to the corresponding indicator in the left atrium, by 174.5% in the right ventricle (RV), and by 142.8% in the left ventricle (LV). Following exposure to chronic hypoxia (CH), this measure in newborn rats significantly increased by 194.7% in RV, by 51.2% in the RV, by 109.7% in the LV, and by 103.3% in the LV compared to the previous term.

The atrial myocardium of animals from the second experimental group was thinner by 74.4% ($p<0.05$) in the RV and by 58.9% ($p<0.05$) in the LV compared to the control group at this specified term. Analyzing the proliferation index of the atrial myocardium in animals from the first experimental group on the 14th day of prenatal development, no significant differences were observed between the proliferation index values of the lateral zones and atria. The maximum value of this indicator was found in the lateral zone of the right atrium - 96±6.02%. The proliferation index values of animals from the first experimental group did not significantly differ from the control group values.

On the first day after the influence of acute hypoxia (AH), the myocardial thickness of rat embryos did not differ in different areas of the atrium. These values did not differ significantly from the control group values in animals from the first experimental group. The proliferation index of the atrial myocardium and auricles on the 16th day of prenatal development in animals from the first experimental group significantly decreased in RV by 52.2%, in the RV by 43.3%, in the LV by 47.4%, and in the LV by 51.4% compared to the previous term. Three days after the influence of AH, these values did not significantly differ from the control group values.

On the 16th day of prenatal development, the thickness values of the atrial and auricular myocardium in animals from the first experimental group did not significantly differ from the values of the previous term and the control group. The myocardial thickness on the 20th day after the influence of AH did not significantly differ compared to the 16th day of prenatal development and compared to the norm. The proliferative activity of atrial cardiomyocytes in animals from the first experimental group gradually decreased until the first day of postnatal development, which was reflected in the reduction in the number of Ki-67 positive cells compared to the 16th day. The proliferation index values of the atrial cardiomyocytes of newborn rats exposed to AH were lower by 49.9% ($p<0.05$) in RV, by 47.4% ($p<0.05$) in the RV, by 34.2% ($p<0.05$) in the LV, and by

39.2% ($p<0.05$) in the LV compared to the previous term, but did not significantly differ from the control group values. On the first day of postnatal development, the myocardial thickness values significantly differed in the right atrial sections: RV - 16.01±2.76 μm, RV - 11.28±1.45 μm. These values in animals from the first experimental group did not significantly differ from the control group values.

The impact of chronic hypoxia (CH) on the proliferative processes of atrial cardiomyocytes was varied: the proliferation index values of animals in the second experimental group at the initial stages of the study (14th day of prenatal development) did not differ from the corresponding normal values. However, by the 16th day of prenatal development, these values were significantly higher, returning to normal values by the first day of prenatal development. Thus, the increase in proliferative activity of atrial cardiomyocytes on the 16th day of prenatal development under the influence of chronic hypoxia appears to be a compensatory response of myocardial cells to the adverse factor. Nevertheless, these changes were transient, gradually approaching normal values by the end of the prenatal period after discontinuation of the CH influence.

In the first experimental group, the proliferation index values of atrial and auricular cardiomyocytes did not significantly differ from the corresponding control group values at all stages of the study, indicating that acute hypoxia does not affect the proliferative processes of atrial cardiomyocytes. We observed an inverse relationship between changes in myocardial thickness and the duration of hypoxia exposure: in animals of the second experimental group on the 14th day of prenatal development (2nd day of hypoxia exposure), the myocardial thickness values did not significantly differ from the norm. However, by the 16th day (4th day of hypoxia exposure), these values were lower by 47.8% ($p<0.05$) in the RV, by 34.8% ($p<0.05$) in the LV, and by 41.4% ($p<0.05$) in the LV compared to the norm. In animals of the first experimental group, the myocardial thickness values did not significantly differ from the norm.

From the 14th day of prenatal development to the first day of postnatal development, under normal conditions and under various hypoxia regimes, the proliferation index of atrial cardiomyocytes tended to decrease, while myocardial thickness gradually increased. Pairwise correlation analysis of these indicators in the control group animals revealed a strong inverse correlation, with a correlation coefficient of 0.69 ($p<0.05$). As previously noted, the influence of chronic hypoxia on the 16th day of prenatal development led to an increase in the proliferation index of cardiomyocytes and a decrease in atrial myocardial thickness in animals of the second experimental group compared to the norm, which resulted in an even higher correlation coefficient 0.86 ($p<0.05$)).

Exposure to hypoxic conditions can elicit a spectrum of alterations and distinct characteristics in the morphological profile of myocardial tissue in rats [24]. Hypoxia, characterized by an insufficient oxygen supply [25], exerts a profound influence on the functionality of the cardiac muscle [26]. The impact of hypoxia includes triggering apoptosisin cardiomyocytes, resulting in the depletion of viable cells and a decline in the count of active muscle fibers [27]. Hypoxia induces structural modifications in cardiomyocytes, manifesting as enlarged cell size and the accumulation of vacuoles within their cytoplasm. It disrupts intercellular connections like desmosomes and their adhesive proteins, potentially disturbing cell-to-cell signaling and coordinated contraction. This detrimental effect impairs myocardial contractility, leading to diminished rhythmic contractions and a subsequent reduction in cardiac output [28]. Interestingly, hypoxia can stimulate angiogenesis, prompting the formation of new blood vessels, as a compensatory mechanism to replenish oxygen delivery to the heart. Hypoxia prompts inflammatory responses within cardiac tissues, influencing the structure and operations of the contractile apparatus [29]. In essence, hypoxia introduces diverse changes in both the structure and functionality of the myocardial contractile apparatus [30], possibly disrupting cardiac function and its efficacy in blood circulation [31-33]. To decipher the intricate mechanisms during hypoxia, in-depth investigations in histology and morphology are imperative [34].

Conclusion

Under conditions of hypoxia and in normal conditions from the 14th day of prenatal development to the 1st day of postnatal development, the myocardial thickness demonstrates an inverse correlation with the proliferation index of atrial cardiomyocytes; under normal conditions, the correlation coefficient is -0.69 ($p<0.05$). When influenced by chronic hypoxia on the 16th day of prenatal development, significant changes in proliferative activity occur: the proliferation index increases, resulting in an even higher correlation coefficient (-0.86 ($p<0.05$)). Acute hypoxia does not significantly affect the processes of proliferation of atrial cardiomyocytes. Prospects for further research. Protects of the research include the application of three-dimensional computer modeling to analyze the morphogenesis of atrial sections of the heart under the influence of various modes of prenatal hypoxia.

Information on conflict of interest

There are no potential or apparent conflicts of interest related to this manuscript at the time of publication and are not anticipated.

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Кобеза П.А. Кількісний та якісний аналіз компонентів міокарда щурів в умовах гіпоксії.

РЕФЕРАТ. Актуальність. Різні види гіпоксії виникають під час внутрішньоутробного розвитку та ранніх неонатальних фаз під впливом факторів, що ускладнюють вагітність. Враховуючи частоту серцево-судинних проблем, розуміння впливу антенатальної гіпоксії на серце новонароджених і вивчення заходів корекції має вирішальне значення для медичних досліджень і практики. **Мета.** Метою дослідження

є аналіз кількісного та якісного складу скорочувальних кардіоміоцитів як первинної клітинної популяції в елементах скорочувального апарату міокарда. Він передбачає вивчення проліферативної варіабельності компонентів скоротливого апарату міокарда в умовах модельованої гіпоксії різного типу. Крім того, він включає кількісний та якісний аналіз клітинного складу елементів міокарда дорослих щурів та їх потомства, які піддавалися різним типам гіпоксії під час внутрішньоутробного розвитку. **Методи.** Дослідження проводили на статевозрілих самках щурів лінії Вістар (4-5,5 місяців) та їх потомстві – новонароджених щурах (14, 16, 20 доба пренатального і 1 день постнатального розвитку). **Результати**. Під впливом хронічної гіпоксії на 14-ту добу внутрішньоутробного розвитку різні ділянки міокарда передсердь демонстрували достовірно різну товщину. Значення цього показника в другій дослідній групі на цьому терміні вірогідно не відрізнялися від значень контрольної групи. Вплив на 16-ту добу внутрішньоутробного розвитку позначився на проліферативній активності. На 16-ту добу внутрішньоутробного розвитку показники товщини міокарда передсердь і передсердь у тварин першої дослідної групи достовірно не відрізнялися від показників попереднього терміну та контрольної групи. Товщина міокарда на 20-ту добу після впливу достовірно не відрізнялася порівняно з 16-ю добу внутрішньоутробного розвитку та порівняно з нормою. Проліферативна активність передсердних кардіоміоцитів у тварин першої дослідної групи поступово знижувалася до першої доби постнатального розвитку, що відобразилося на зменшенні кількості Кі-67 позитивних клітин порівняно з 16-ю добою. **Підсумок.** В умовах гіпоксії та в нормі з 14-ї доби внутрішньоутробного розвитку до 1-ї доби постнатального розвитку товщина міокарда демонструє зворотну кореляцію з індексом проліферації кардіоміоцитів передсердь; в нормальних умовах. Гіпоксія істотно не впливає на процеси проліферації кардіоміоцитів передсердь.

Ключові слова: тканинна гіпоксія, кардіоміоцит, скоротливий апарат, ультраструктура, міофібрили.