Original article

Development of the Heart Muscle after Antenatal Ethanol Intoxication during the Neonatal Period

Galina Zhanaidarova[1], Nurlan Nauryzov[1], Kulash Nurseitova[1], Leila Arystan[2], Rustam Dyussembekov[3], Gulmira Turdunova[4], Sayn Zhanbyrbaeva[3], Gulbanu Akhmetova[1]*

Abstract

Objective: The aim of the study was to study the effect of antenatal ethanol intoxication on the postnatal development of the heart muscle during the neonatal period. ***Materials and methods:*** The experiment was carried out on 24 pregnant female rats weighing 180-200 grams. The animals were divided into 2 groups: experimental and control. 12 animals of the experimental group were injected with a 30% ethanol solution from 6 to 21 days of gestation daily, once, endogastrically, at a dose of 5 g / kg. The animals of the control group were injected with the same volume of physiological saline. ***Results and Discussion:*** On the 1st and 7th days after birth, the rat pups were killed by decapitation under light ether anesthesia. After determining the mass of the heart, part of the organ was studied electron microscopically, part was fixed in 10% neutral formalin, then histological preparations were prepared in accordance with standard methods. Sections were stained with hematoxylin and eosin. The nuclear-cytoplasmic ratio in cardiomyocytes, the specific density of the capillary network of the myocardium, and the specific density of cardiomyocytes were measured. The data obtained were statistically processed. ***Conclusion:*** The study showed that antenatal ethanol intoxication has a pronounced effect on the postnatal development of the heart muscle during the neonatal period, which manifests itself at the cellular and subcellular levels. The value of the obtained results lies in the fact that for the first time ultrastructural changes in postnatal development in the neonatal period of the fetal heart muscle during prenatal ethanol intoxication are shown at the electron microscopic level.

Keywords: cardiac muscle; electron microscopy; fetal alcohol syndrome; neonatal period; postnatal development.

Bangladesh Journal of Medical Science Vol. 21 No. 02 April'22 Page : 344-353
DOI: http://doi.org/10.3329/bjms.v21i2.58067

Introduction

The study of the problem of alcoholism is relevant.[1-4] Alcoholism affects the central nervous system,[5,6] cardiovascular,[7,8] and digestive systems.[9,10] Female alcoholism is especially dangerous, as its consequence is the birth of children with fetal alcohol syndrome (FAD).[11,12] FAD is characterized by a combination of facial dysmorphism and various intrauterine malformations, among which heart defects are often found.[13-15] It is well known that the placenta easily passes ethanol from the mother's blood into the fetal blood; ethanol is also found in the amniotic fluid, therefore, the toxic substance affects the development of tissues, organs and systems of the fetus.

The antenatal period is one of the most key periods in the development of the body and its functions,

1. Department of Morphology and Physiology, Non-Profit Joint Stock Company "Medical University of Karaganda", Karaganda, Kazakhstan
2. Department of Family Medicine, Non-Profit Joint Stock Company "Medical University of Karaganda", Karaganda, Kazakhstan
3. Department of Neurology, Neurosurgery, Psychiatry and Rehabilitation, Non-Profit Joint Stock Company "Medical University of Karaganda", Karaganda, Kazakhstan
4. Department of Obstetrics and Gynecology, Non-Profit Joint Stock Company "Medical University of Karaganda", Karaganda, Kazakhstan

Correspondence: Galina Zhanaidarova, Department of Morphology and Physiology, Non-Profit Joint Stock Company "Medical University of Karaganda", Karaganda, Kazakhstan, e-mail: gzhanaidarova@rambler.ru

which determines health throughout its subsequent life. It has been established that the offspring who have undergone antenatal ethanol intoxication, after birth, have various disorders of the development of organs, which can be noted already in the neonatal period. In the experiment, antenatal ethanol intoxication causes damage to the membranes of the cells of the cerebral cortex in newborn rat pups.[16]Antenatal ethanol intoxication causes changes in the liver and lymphoid tissues of newborn rats associated with oxidative stress and damage to the membranes of liver cells and lymphoid tissues.[17,18] Experimentally, on the offspring of 5-day-old mice, it was found that prenatal intoxication with ethanol affects the function of fibroblasts, contributing to the dysregulation of collagen synthesis, which leads to heart dysfunction.[13,19]

A research gap is the extremely insufficient number of morphological works devoted to the features of postnatal heart development after antenatal ethanol intoxication.

The contribution of this article to world science lies in the fact that for the first time an electron microscopic study of the heart muscle was carried out in the neonatal period after antenatal intoxication with ethanol.

It has been shown that antenatal ethanol intoxication is a factor causing abnormalities in the postnatal development of the myocardium. On the 1st day after birth, there is a decrease in the level of differentiation of muscle cells, expressed in the weak development of the myofibrillar apparatus, intercellular contacts, up to the absence of direct intercalated discs characteristic of this period. Basically, intercellular connections were represented by the embryonic type of simple intercellular contacts. 7 days after birth, the degree of differentiation of muscle cells remains reduced, which is manifested in a weak development of the sarcoplasmic reticulum, a sharp decrease in full-fledged cell junctions, a pronounced expansion of poorly developed intercalated discs.

The aim of our study was to study the effect of antenatal ethanol intoxication on the postnatal development of the heart muscle during the neonatal period.

Methods

The experiment was carried out on 24 white rats, weighing 180-200 grams, which were kept in the usual conditions of the vivarium. The first day of pregnancy was considered the day of detection of sperm in vaginal smears. The animals were divided into 2 groups: experimental and control. 12 animals of the experimental group were injected with a 30% ethanol solution from 6 to 21 days of gestation daily, once, endogastrically, at a dose of 5 g / kg. The animals of the control group were injected in the same volume with physiological saline. After birth, the postnatal development of the offspring was studied in both groups. On the 1st and 7th day, the pups were slaughtered by decapitation under light ether anesthesia. After determining the mass of the heart, a part of the organ was fixed in 10% neutral formalin, then histological preparations were prepared in accordance with standard methods. The material was poured into a Histomix paraffin medium, then 5 micrometers thick sections were cut on an MC-2 microtome using Accu-Ebge®35 disposable microtome knives for routine sections. Sections were stained with hematoxylin and eosin. To study the sections, we used a medical laboratory binocular microscope of the Micros series, model MC20, manufactured in Austria. The nuclear-cytoplasmic ratio in cardiomyocytes, the specific density of the capillary network of the myocardium, and the specific density of cardiomyocytes were measured. To assess the histological preparations, a morphometric method was used: determination of the relative number of individual structures per unit area using an eyepiece grid according to Avtandilov.[20] The data obtained were statistically processed. For electron microscopic examination, part of the material taken (cardiac muscle) was fixed in a 2.5% glutaraldehyde solution in 0.2M Millonig's phosphate buffer (pH 7.4). Semi-thin sections, 1-3 μm thick, were prepared on a Tesla ultramicrotome. Semi-thin sections were stained with methylene blue - azure II and basic fuchsin. In this case, glycogen inclusions were stained in raspberry, and fat drops in olive green. Ultrathin sections were prepared using LKB and Reichert ultramicrotomes, then they were contrasted with uranyl acetate and lead citrate. Observations and filming of ultrathin tissue sections were performed using an EVM-100 electron microscope.

Data, Analysis and Results

Our study showed that antenatal ethanol intoxication in experimental animals in the postnatal period lags behind in the dynamics of the increase in heart mass both on days 1 and 7 of the study (Table 1).

Table 1. Dynamics of changes in the heart weight of rat pups on days 1 and 7 of postnatal development (mg)

Experimental conditions Timing of the experiment	the control	ethanol
1 day of postnatal development	41.64±2.165	37.52± 1.838
7 days of postnatal development	71.24±3.491	58.31± 3.265

The study of the nuclear-cytoplasmic ratio (NCR) of cardiomyocytes showed that in control animals this indicator significantly decreases on the 7th day. The same pattern is observed in experimental animals, but when comparing these indicators at the same time, one can see that the intensity of the decrease in control animals is higher, which indicates more active processes of muscle cell differentiation (Table 2).

Table 2. Dynamics of NCR changes in myocardial cardiomyocytes of rat pups on days 1 and 7 of postnatal development (a.u.)

Experimental conditions Timing of the experiment	the control	ethanol
1 day of postnatal development	0.152±0.005	0.167±0.006
7 days of postnatal development	0.138±0.068	0.156±0.006

The study of the dynamics of changes in the specific density of myocardial cardiomyocytes of rat pups showed that in control animals this indicator on the 1st and 7th days of life exceeds the indicators of the experimental group, which indicates more active processes of differentiation of muscle cells and growth of the heart muscle (Table 3).

Table 3. Dynamics of changes in the specific density of myocardial cardiomyocytes in rat pups on days 1 and 7 of postnatal development (a.u.)

Experimental conditions Timing of the experiment	the control	ethanol
1 day of postnatal development	62.02 ± 2.977	59.99 ± 1.558
7 days of postnatal development	64.29 ± 2.314	59.97 ± 1.379

The study of the dynamics of changes in the specific density of the capillary network of the myocardium of rat pups showed that in control animals this indicator increases with their growth, exceeding the indicators of the experimental group on the 1st and 7th days of life, which indicates more active processes of development of the capillary network, and, consequently, the creation of more favorable trophic conditions for cardiac muscle cells (Table 4).

Table 4. Dynamics of changes in the specific density of the capillary network of the myocardium of rat pupson the 1st and 7th days of postnatal development (a.u.)

Experimental conditions Timing of the experiment	the control	ethanol
1 day of postnatal development	29.33 ± 1.232	24.82 ± 0.719
7 days of postnatal development	29.84 ± 1.104	25.64 ± 0.795

Electron microscopic examination of control animals revealed that 1 day after birth, the myocardium was represented mainly by cardiomyocytes and occasionally by cardiopromiocytes. Electronomicroscopically, the cardiopromiocytes had an elongated shape and were located loosely. Whole fields occupied by RNA and glycogen granules were visible in the cytoplasm. In the center there were beams of protofibrils with Z - lines. Cardiomyocytes were characterized by the presence of a large nucleus of an oblong or irregular shape with granular chromatin located primarginally and in some parts of the karyoplasm. The nuclear envelope had deep invaginations. The nucleolus is large, granular. The perinuclear space is narrow or slightly enlarged (Figure 1).

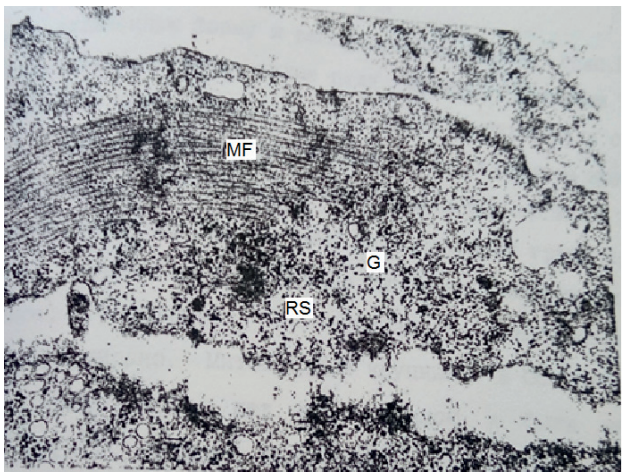

Figure 1. 1 day. The control. Postnatal development. In the cytoplasm of the cardiopromiocyte, myofibrils with Z - lines (MF), numerous glycogen granules (G) and ribosomes (RS) are visible. Electronogram. x24000.

Mitochondria are large, mostly located near the nucleus, under the plasmolemma, and were numerous. Dividing mitochondria were occasionally

seen. In the enlightened matrix, myelin-like structures were encountered, which were formed as a result of increased energy metabolism and disruption of peroxidation processes. Myofibrils had a characteristic cross striation. The cytoplasm was rich in RNA and glycogen granules. Poorly developed longitudinal and transverse systems of the sarcoplasmic reticulum are noted. Cell junctions were represented by fully developed straight intercalated discs (Figure 2).

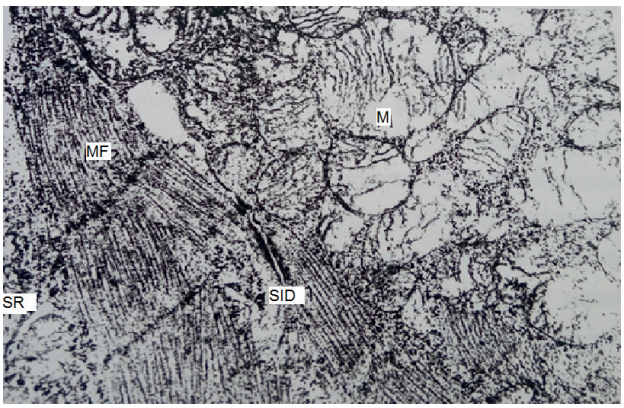

Figure 2.1 day. The control. Postnatal development. In the cytoplasm of the cardiomyocyte, numerous mitochondria (M), bundles of myofibrils (MF), vesicles of the sarcoplasmic reticulum (SR) are visible. SID - straight intercalateddiscs. Electronogram. x18000.

The endothelium of the capillaries was distinguished by a significant thickness, a high content of organelles and pinocytic vesicles (Figure 3). The apical surface was smoothed or contained long microvilli. Cell junctions were of a simple type. Rarely occurring pericytes were also characterized by good development of the cytoplasmic reticulum, lamellar complex, free ribosomes, and pinocytic vesicles.

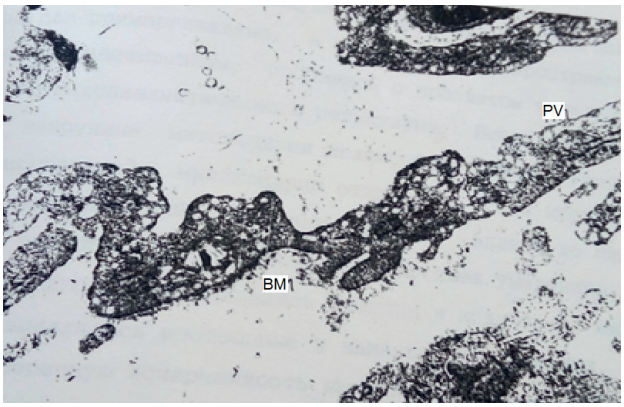

Figure 3.1 day. The control. Postnatal development. The endothelium of the capillary is thickened. PV - pinocytic vesicles, BM - basement membrane. Electronogram. x18 000.

When studying the ultrastructure of the myocardium in offspring with antennae intoxication with ethanol, it was found that 1 day after birth, cardiomyocytes and cardiopromiocytes of the heart muscle were characterized by phenomena of intracellular edema with clearing of the hyaloplasm, lysosomes and myelination of mitochondrial cristae, a sharp decrease in the number of myofibrils (Figure 4).

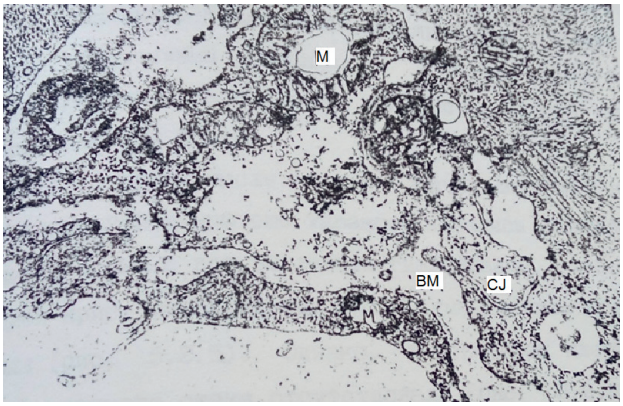

Figure 4.1 day. Antenatal ethanol intoxication. Postnatal development. Cardiopromiocyte. Intracellular edema, myelination of mitochondrial cristae (M), absence of myofilaments. Simple cell junctions (CJ). In the endothelium of the capillary - loosening of the basement membrane (BM). Electronogram. x18000.

However, in some cells there were no signs of intracellular edema. They were characterized by the presence of a large nucleus of irregular, occasionally lobed, shape with uniformly distributed ribonucleoprotein granules. The perinuclear space was narrow. The cytoplasm contained numerous polymorphic mitochondria with vacuolated and myelinated cristae. In some mitochondria, the cristae were completely lysed. The tubules of the sarcoplasmic reticulum were poorly developed and sharply expanded. The amount of glycogen granules was reduced. Myofibrils are poorly developed, the arrangement of bundles of myofibrils is disordered (Figure 5).

We also noted completely immature muscle cells of the myoblast type, in the cytoplasm of which individual myofilaments were located. These cells were distinguished by a poorly developed granular endoplasmic reticulum, the presence of small electron-dense mitochondria, a small number of free ribosomes, and the absence of glycogen inclusions. The cells were characterized by simple intercellular contacts characteristic of embryonic development. In

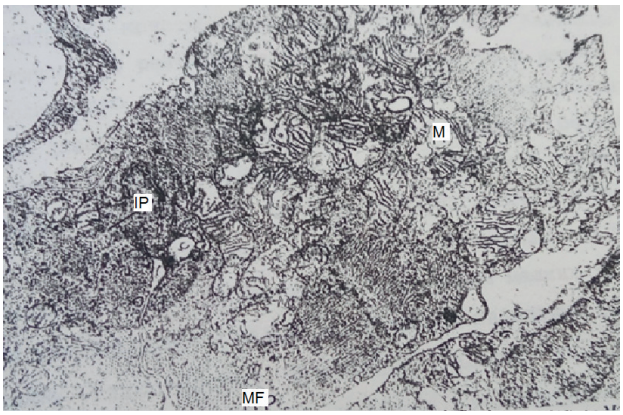

Figure 5.1 day. Antenatal ethanol intoxication. Postnatal development. Myelination, lysis of mitochondrial cristae (M). Rare intercellular plates are visible (IP). MF - myofibrils. Electronogram. x10 000

the expanded intercellular space, numerous lamellar and homogeneous structures were visible.

A decrease in the level of differentiation of muscle cells was also seen by poorly developed cell junctions, the absence of complicated intercalated discs, which are observed in the norm during this period of development (Figures 4 and 5). The endothelium of the capillaries was sometimes distinguished by a sharp tortuosity of the apical surface with the appearance of long micro-processes. Both in the cytoplasm of endothelial cells and pericytes, the phenomena of swelling of mitochondria, lysis of cristae, and sharp vacuolization of the reticulum were noted. In the interstitial space, there were still signs of intercellular edema, the presence of a large amount of flocculent material around the capillaries.

Thus, on the 1st day of postnatal development after antenatal intoxication with ethanol, despite the compensatory - adaptive reactions in the cardiomyocytes and cardiopromiocytes of the heart muscle, there were still signs of intra- and intercellular edema. Mitochondrial hyperplasia was accompanied by pronounced destructive changes in the inner mitochondrial membrane. It is important to emphasize the decrease in the level of differentiation of muscle cells, expressed in the weak development of the myofibrillar apparatus,cell junctions, up to the absence of normal intercalated discs characteristic of this period. Basically, cell junctions were represented by the embryonic type of simple intercellular contacts.

In the control group, 7 days after birth, cardiomyocytes were observed in the myocardium. They possessed a very large oval core with wavy or scalloped contours of the nuclear envelope. Depending on the activity of protein synthesis, granular chromatin was distributed uniformly or primarginal, and the perinuclear space was narrow or widened, communicating with the lumen of the tubules of the granular endoplasmic reticulum. Small polymorphic mitochondria were located near the nucleus. The mitochondria located under the sarcolemma were distinguished by their larger sizes, up to giant, irregular lobed shape. The crystals were densely packed, the matrix had a moderate electron density. The thickness and number of myofibrils increased. Isotropic and anisotropic areas were well identified, determining the transverse striation of muscle fibers. The sarcoplasmic reticulum included a more distinct longitudinal and transverse tubular system.In the sarcoplasm, vacuolar and membrane components of the Golgi complex, free ribosomes and glycogen granules were noted. In the area of Z - lines, the sarcolemma formed arched protrusions. The intercalated disks crossed the muscle fiber in the transverse direction and looked like a curved line, occasionally with deep invaginations of the plasma membrane (Figure 6).

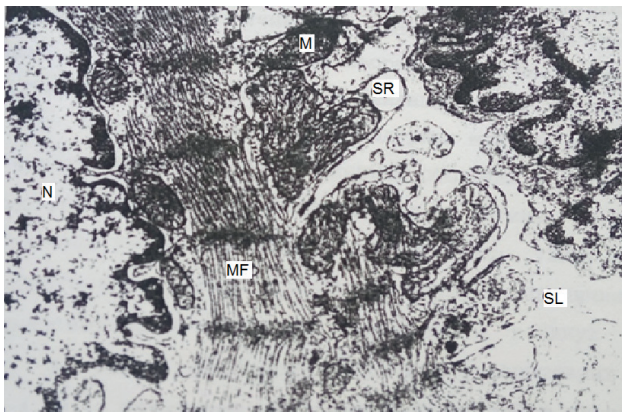

Figure 6. 7 days. The control. Postnatal development. Cardiomyocyte. N - nucleus, MF - myofibrils, M - mitochondria, SR - sarcoplasmic reticulum, SL - sarcolemma. Electronogram. x18000

The capillary endothelium contained a large oval nucleus with wavy contours of the nuclear envelope and a primarginal distribution of condensed chromatin. A moderate number of organelles was noted in the cytoplasm: narrow tubules of the granular endoplasmic reticulum, single large electron-light mitochondria, membranes and vesicles of the Golgi complex, as well as free ribosomes and pinocytosis vesicles. The basement membrane was loose and poorly expressed (Figure 7).

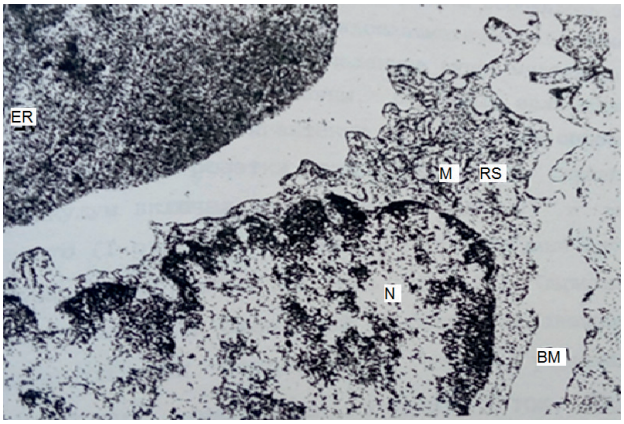

Figure 7. 7 days. The control. Postnatal development. Capillary endothelium. N - nucleus, M - mitochondria, RS - ribosomes, BM - basement membrane, ER - erythrocyte. Electronogram. X24000.

In the experimental group, 7 days after birth, cardiomyocytes contained a large nucleus with scalloped indented contours of the nuclear envelope and a primarginal distribution of condensed chromatin. The perinuclear space was slightly and unevenly expanded. The cytoplasm contained large, bizarre lobed shape, electron-dense mitochondria with a large number of densely packed cristae and a matrix of increased electron density. The sarcoplasmic reticulum was poorly developed, its vesicles were expanded. In the hyaloplasm, there were still areas of local intracellular edema, especially along the periphery of the cells with the appearance of many pinocytic vesicles and sarcolemma detachment. The glycogen content has been reduced. Full contacts in the form of convoluted intercalated discs were rare. The intercellular space in the region of the existing weakly developed intercalated disks was sharply expanded (Figure 8).

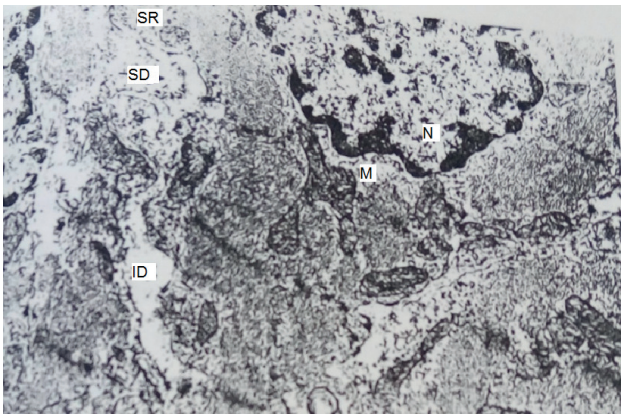

Figure 8. 7 days. Antenatal ethanol intoxication. Postnatal development. Formation of giant mitochondria (M). Edema, poor development of the sarcoplasmic reticulum (SR), sarcolemma detachment (SD). The distance between the intercalated discs is sharply widened (ID). N – nucleus. Electronogram. x16 800

Some cardiomyocytes were in a state of partial necrosis and destruction. At the same time, destructively altered organelles and the remains of lysed myofibrils were visible in the cytoplasm. The endothelium of the capillaries contained large nuclei, occupying almost the entire volume of the central part of the endothelium, with 1 - 2 nucleoli and a primarginal distribution of condensed chromatin. The cytoplasm of endothelium and pericytes was characterized by increased density due to the abundance of ribosomes, narrow short cisterns of the reticulum. A pronounced compensatory reaction of an increase in the apical and basal surface of capillary cells was noted due to the appearance of numerous long outgrowths, as if penetrating the basement membrane. The basement membrane was thickened, loosened, reduplicated, and absent in some areas (Figure 9).

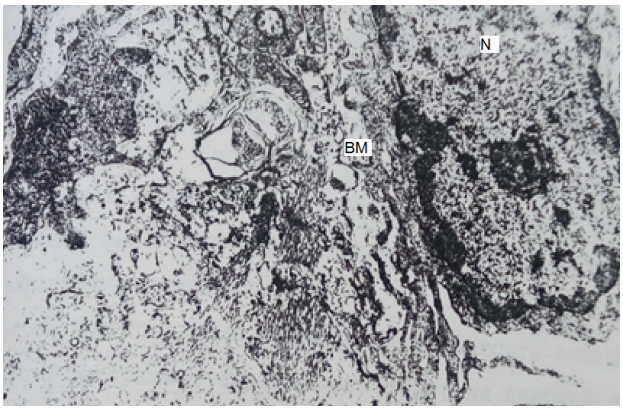

Figure 9. 7 days. Antenatal ethanol intoxication. Postnatal development. Destruction of a cardiomyocyte. N- the nucleus of the capillary endothelium. Numerous cytoplasmic outgrowths of the apical and basal surfaces of the capillary endothelium. The basement membrane (BM) is fragmented and loosened. Electronogram. x18000.

Thus, 7 days after birth in the experimental group, against the background of still existing signs of dystrophy, destruction and edema of cardiomyocytes, compensatory - adaptive reactions increased, manifested in an increase in nuclear protein synthesis, expressed intracellular regeneration of mitochondria. However, the degree of differentiation of muscle cells was reduced, which was manifested in a weak development of the sarcoplasmic reticulum, a sharp decrease in full-fledged cell junctionts, a pronounced expansion of poorly developed intercalated discs.

Discussion

In the process of early postnatal ontogenesis of rats, four critical periods are distinguished: birth, neonatal period (early milk period) - the first week after birth, the period of eye opening (14–20 days) and the period of transition to independent feeding - 28 days.[21,22]

An experimental study of the neonatal period showed that antenatal intoxication with ethanol causes a delay in the development of rat pups on days 1 and 7, which is manifested by a decrease in heart weight (Table 1) relative to control animals. The growth retardation of organs in fetal alcohol syndrome is affected by a decrease in tissue sensitivity to hormones that control growth.[23] In children who have undergone antenatal ethanol intoxication, there is a tendency to an increase in the concentration of transforming growth factor in the blood, which indicates a deficiency of receptors for this growth factor, associated with exposure to ethanol, which may result in growth retardation in the postnatal period.[24]

The decrease in the mass of the heart is associated with the processes occurring in the heart muscle. Electron microscopic examination showed that on day 1 after birth, the myocardium of the control animals contained muscle cells of varying degrees of differentiation: cardiomyocytes and single cardiopromiocytes.In the group with antenatal ethanol intoxication, in addition to them, there were also completely immature muscle cells of the myoblast type, in the cytoplasm of which individual myofilaments were located. This indicates a delay in the postnatal development of the myocardium, since in the control these cells of the myoblast type are absent due to the fact that they managed to differentiate into cardiopromiocytes and cardiomyocytes. On the 7th day, the heart muscle consists only of cardiomyocytes.

Since ethanol intoxication is removed after birth, compensatory-adaptive reactions begin in the myocardium of experimental animals. However, they proceed in stages. On the 1st day after birth, in the experimental group, compared with the control, significant ultrastructural changes were noted. In cardiomyocytes and cardiopromiocytes of the heart muscle, there are signs of intra- and intercellular edema. Mitochondrial hyperplasia was accompanied by pronounced destructive changes in the inner mitochondrial membrane, up to complete lysis of the cristae. The tubules of the sarcoplasmic reticulum were poorly developed and sharply expanded. The bundles of weakly developed myofibrils were arranged randomly.

There was a decrease in the level of differentiation of muscle cells, expressed in the weak development of the myofibrillar apparatus, intercellular contacts, up to the absence of normal intercalated discs characteristic of this period. Basically, cell junctions were represented by the embryonic type of simple intercellular contacts. We regard these ultrastructural changes as caused by antenatal ethanol intoxication. This is supported by literature data, from which it is known that the etiology of cardiotoxicity associated with ethanol is multifactorial. Ethanol causes apoptosis, changes in the interaction of excitation and contraction in cardiac myocytes, structural and functional changes in mitochondria and sarcoplasmic reticulum, changes in cytosolic calcium fluxes, changes in the calcium sensitivity of myofilaments, changes in mitochondrial oxidation, deregulation of protein synthesis, a decrease in contractile proteins and disproportions between different types of myofibers changes in the regulation of myosin-ATPase, increased regulation of L-type calcium channels, increased oxidative stress and induction of ANP and p21 mRNA expression in the ventricular myocardium.[25]

The targets for ethanol in myocytes are membranes, receptors, ion channels, intracellular migration of Ca ions, structural proteins, which disrupts the contractility of the sarcomere.[26] In our case, it is with the changes in the membrane structure under the influence of ethanol that destructive changes in the internal mitochondrial membranes, which are reflected in the bioenergetics of cardiomyocytes, can be associated; membranes of the sarcoplasmic reticulum associated with intracellular migration of Ca ions. The same applies to cell membranes that form intercellular contacts. Ethanol, naturally, acts not only on the membranes of cardiomyocytes, but also on the membranes of other cells in the myocardium, for example, on endothelial cells and capillary pericytes, which form the capillary bed of the myocardium. Electron-microscopically, it was established that on day 1 after birth in the cytoplasm of the latter, the phenomena of swelling of mitochondria, lysis of cristae, and sharp vacuolization of the reticulum were noted. This alcoholic lesion of endothelial cells and

pericytes undoubtedly slows down the development of the capillary network and explains the lag of these indicators in the experimental group from the control one both by day 1 and by 7 (Table 4).

7 days after birth in the experimental group, individual cardiomyocytes were in a state of partial necrosis and destruction. At the same time, destructively altered organelles and the remains of lysed myofibrils were visible in the cytoplasm. Against the background of still existing signs of dystrophy, destruction and edema of cardiomyocytes, compensatory - adaptive reactions intensified, manifested in an increase in nuclear protein synthesis, pronounced intracellular regeneration of mitochondria.However, the degree of differentiation of muscle cells was reduced, which was manifested in a weak development of the sarcoplasmic reticulum, a sharp decrease in full-fledged cell junctions, a pronounced expansion of poorly developed intercalated discs. In the capillaries of the myocardium, the cytoplasm of the endothelium and pericytes was distinguished by an increased density due to the abundance of ribosomes, narrow short cisterns of the reticulum. A pronounced compensatory reaction of an increase in the apical and basal surface of capillary cells was noted due to the appearance of numerous long outgrowths, as if penetrating the basement membrane. The basement membrane was thickened, loosened, reduplicated, and absent in some areas.

The decrease in differentiation of cardiomyocytes after antenatal intoxication with ethanol is confirmed by the data obtained by us in the study of the NCR of cardiomyocytes, the dynamics of changes in the specific density of cardiac muscle cells in the offspring of rats in the neonatal period, and the dynamics of changes in the specific density of the capillary network of the rat myocardium.The study of the NCR of procardiomyocytes and cardiomyocytes showed that in control animals on the 1st and 7th day this indicator is lower than in the experimental group, which indicates more active processes of muscle cell differentiation (Table 2). The study of the dynamics of changes in the specific density of cardiac muscle cells in the offspring of rats during the neonatal period showed that on days 1 and 7 in control animals this indicator is higher than in animals of the experimental group, which indicates more active processes of differentiation of muscle cells and growth of cardiac muscle (Table 3).

We determined the density of the capillary bed of the myocardium on the 1st and 7th days of postnatal development, which are periods of active functional development of the capillary bed. It was revealed that the density of the capillary bed after antenatal alcohol intoxication lags behind the indicators of control animals (Table 4). This slow rate of increase in the density of the capillary bed can be associated in the postnatal period with inhibition of the functional activity of endothelial cells and pericytes, due to the disorganization of metabolic processes and energy supply of cells by alcohol. Naturally, in the altered endotheliocytes, transport processes, their synthetic and plastic functions will be disrupted.[27] Our data are consistent with the results of clinical studies that showed that under conditions of prenatal alcoholization, there is a decrease in the diameter and perimeter of capillaries with an increase in the period of development, leading to a general decrease in tissue vascularization.[28,29]

The results concerning the delay in the postnatal development of the offspring that underwent antenatal ethanol intoxication are reliable. This is confirmed by the data of statistical processing of our material, similar results obtained in the clinic and experiment,[25,26,30] as well as obtained by us electron microscopic data.

The results obtained in the course of the study are of undoubted value, since the electron microscopic data obtained by us are new data in the structure of knowledge known to mankind.

Conclusion

The article presents valuable scientific data previously unknown. Electron microscopic examination revealed that antenatal intoxication with ethanol on the 1st day of postnatal development causes a decrease in the level of differentiation of muscle cells, expressed in the weak development of the myofibrillar apparatus, intercellular contacts, up to the absence of direct intercalated discs typical for this period. Basically, cell junctions were represented by the embryonic type of simple intercellular contacts. We observed completely immature muscle cells of the myoblast type, in the cytoplasm of which individual myofilaments were located. Signs of intra- and intercellular edema were observed in

cardiomyocytes and cardiopromiocytes of the heart muscle. Mitochondrial hyperplasia was accompanied by pronounced destructive changes in the inner mitochondrial membrane. It was found that on the 7th day of the postnatal period, against the background of still existing signs of dystrophy, destruction and edema of cardiomyocytes, compensatory - adaptive reactions increased, manifested in an increase in nuclear protein synthesis, pronounced intracellular regeneration of mitochondria. However, the degree of differentiation of muscle cells was reduced, which was manifested in a weak development of the sarcoplasmic reticulum, a sharp decrease in full-fledged cell junctions, and a pronounced expansion of poorly developed intercalary discs.

Antenatal ethanol intoxication at the cellular level in the postnatal period is manifested by a delay in differentiation of cardiomyocytes, a decrease in the rate of increase in their specific density and the specific density of the myocardial capillary network in rat offspring during the neonatal period.

Funding. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interests. Authors declare that they have no conflict of interests.

Data Availability. Data will be available on request.

Ethical clearance. The authors declare that the work is written with due consideration of ethical standards. The study was conducted in accordance with the ethical principles approved by the Ethics Committee of Non-Profit Joint Stock Company «Medical University of Karaganda» (Protocol № 7 of 17.04.2020).

Authors's contribution.

Data gathering and idea owner of this study: GZ, NN, RD, and SZ

Study design: NN, KN, LA, RD, GT, and GA

Data gathering: NN, LA, RD, GT, and SZ.

Writing and submitting manuscript: GZ, KN, GT, and GA

Editing and approval of final draft: GZ, KN, LA, SZ, and GA

References

1. Ishiguro H, Higuchi S, Arinami T and Onaivi ES. Association between alcoholism and the gene encoding the endocannabinoid synthesizing enzyme diacylglycerol lipase alpha in the Japanese population. *Alcohol* 2018; 68: 59-62. https://doi.org/10.1016/j.alcohol.2017.09.005
2. Kumar R, Kumar KJ, Benegal V, Roopesh BN and Ravi GS. Effectiveness of an Integrated Intervention Program for Alcoholism (IIPA) for enhancing self-regulation: Preliminary evidence.*Asian J Psychiatr*.2019; 43: 37-44. https://doi.org/10.1016/j.ajp.2019.05.006
3. Huo HF, Cui FF and Xiang H. Dynamics of an SAITS alcoholism model on unweighted and weighted networks. *Physica A Stat Mech Appl*.2018; 496: 249-262. https://doi.org/10.1016/j.physa.2018.01.003
4. Huo HF, Xue HN and Xiang H. Dynamics of an alcoholism model on complex networks with community structure and voluntary drinking. *Physica A Stat Mech Appl*.2018; 505: 880-890. https://doi.org/10.1016/j.physa.2018.04.024
5. Liu Q, Yan L, Huang M, Zeng H, Satyanarayanan SK, Shi Z et al. Experimental alcoholism primes structural and functional impairment of the glymphatic pathway. *Brain Behav Immun*.2020; 85: 106-119. https://doi.org/10.1016/j.bbi.2019.06.029
6. Kirson D, Oleata CS and Roberto M. Taurine suppression of central amygdala GABAergic inhibitory signaling via glycine receptors is disrupted in alcohol dependence. *Alcohol Clin Exp Res*.2020; **44**(2): 445-454. https://doi.org/10.1111/acer.14252
7. Wang S and Ren J. Role of autophagy and regulatory mechanisms in alcoholic cardiomyopathy. *Biochim Biophys Acta Mol Basis Dis*.2018; **1864**(6): 2003-2009. https://doi.org/10.1016/j.bbadis.2018.03.016
8. Jahan N, Mannan MA, Akter S, Afroz F, Farhana T, Nasrin M et al. Prematurity: A major cause of early neonatal mortality in Ad-din Medical College Hospital. *Bangladesh J Med Scie*.2019; **18**(3): 593-597. https://doi.org/10.3329/bjms.v18i3.41634
9. Testino G, Vignoli T, Patussi V, Scafato E and Caputo F. Management of end-stage alcohol-related liver disease and severe acute alcohol-related hepatitis: position paper of the Italian Society on Alcohol (SIA). *Dig Liver Dis* 2020;**52**(1): 21-32. https://doi.org/10.1016/j.dld.2019.09.017
10. Sultana M, Jahan N, Sultana N and Doha M. Low level of serum Hemoglobulin concentration associated with neonatal birth weight. *Bangladesh J Med Sci* 2020; **19** (2): 278-283. https://doi.org/10.3329/bjms.v19i2.45008
11. Popova S, Lange S, Probst C, Gmel G and Rehm J. Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. *Lancet Glob Health*. 2017; **5** (3): 290-299. https://doi.org/10.1016/S2214-109X(17)30021-9
12. May PA, Chambers CD, Kalberg WO, Zellner J, Feldman H, Buckley D et al. Prevalence of fetal alcohol spectrum disorders in 4 *US communities*. *Jama* 2018;**319**(5): 474-482. https://doi.org/10.1001/jama.2017.21896
13. Singal N, Taneja BK, Setia G and Singal KK. Foetal outcome in pregnant women with anaemia. *Bangladesh J Med Sci*.2018; **18** (1): 63-72. https://doi.org/10.3329/bjms.v18i1.39551
14. Zhang S, Wang L, Yang T, Chen L, Zhao L, Wang T et al. Parental alcohol consumption and the risk of congenital heart diseases in offspring: An updated systematic review and meta-analysis. *Eur J Prev Cardiol* 2020;**27**(4): 410-421. https://doi.org/10.1177/2047487319874530
15. Krichevskaya GI, Slepova OS, Sahakyan SV, Myakoshina EB and Denisova YV. On the role of infections in the pathogenesis of retinoblastoma. *Russian Ophthalmological Journal*.2013;**6** (2): 37-40.
16. Sogut I, Oglakci A, Kartkaya K, Ol KK, Sogut MS, Kanbak G et al. Effect of boric acid on oxidative stress in rats with fetal alcohol syndrome. *Exp Ther Med*.2015; **9**(3): 1023-1027. https://doi.org/10.3892/etm.2014.2164
17. Ince E. The protective effect of quercetin in the alcohol-induced liver and lymphoid tissue injuries in newborns. *MolBiol Rep*.2020; **47** (1): 451-459. https://doi.org/10.1007/s11033-019-05148-0
18. Saakyan SV, Myakoshina EB, Yurovskaya NN and Ryabina MV. Metastatic carcinoma of chorioida: two clinical cases. *Bulletin of Ophthalmology*.2013; **129** (2): 56-60.
19. Ninh VK, El Hajj EC, Mouton AJ and Gardner JD. Prenatal alcohol exposure causes adverse cardiac extracellular matrix changes and dysfunction in neonatal mice. *Cardiovasc Toxico*.l2019; **19**(5): 389-400. https://doi.org/10.1007/s12012-018-09503-8
20. Avtandilov GG. Medical morphometry. Manual. Moscow, Medicine, 1990.
21. Neroyev VV, Saakian SV, Miakoshina EB, Iurovskaia NN, Riabina MV, Zaĭtseva OV et al. Differential diagnosis of early central choroidal melanoma and late stage age-related macula degeneration. *Vestnik Oftalmologii* 2013;**129(**1):39-45.
22. Zicha J and Kunes J. Ontogenetic aspects of hypertension development: analysis in the rat. *Physiol Rev* 1999; **79** (4): 1227-1282. https://doi.org/10.1152/physrev.1999.79.4.1227
23. Castells S, Mark E, Abaci F and Schwartz E. Growth retardation in fetal alcohol syndrome. 1981;3: 232-241. https://doi.org/10.1159/000457447
24. Saakyan SV, Zharua AA, Myakoshina EB, Lazareva LA and Yakovlev SB. Superselective intra-arterial chemotherapy treatment for resistant and severe retinoblastoma. *Russian Pediatric Ophthalmology*. 2013; 1: 31-34.
25. Hantson P. Mechanisms of toxic cardiomyopathy. *Clin Toxicol*.2019;**57** (1): 1-9. https://doi.org/10.1080/15563650.2018.1497172
26. Fernandez-Sola J. The effects of ethanol on the heart: Alcoholic cardiomyopathy. *Nutrients* 2020; **12** (2): 572. https://doi.org/10.3390/nu12020572
27. Ilyicheva VN, Sokolov DA, Nasonova NA, Karandeeva AM and Minasyan VV. The influence of alcohol in various doses on the permeability of the blood-brain barrier. InActual problems of medical science and education (APMNO-2019): a collection of articles based on the materials of the VII International Scientific Conference dedicated to the 80th anniversary of the Penza region and the 20th anniversary of the PSU Medical Institute.Penza, Penza State University (Penza), 2019: 27-29.
28. Yumashev AV, Gorobets TN, Admakin OI, Kuzminov GG and Nefedova IV. Key aspects of adaptation syndrome development and anti-stress effect of mesodiencephalic modulation. *Indian J Sci Technol*. 2016; **9** (19): 93911. https://doi.org/10.17485/ijst/2016/v9i19/93911
29. Shumilova SN, Potapov AV and Logvinov SV. Characteristics of morphological and quantitative changes in the vessels of the microvasculature and neurons in the brain of embryos under conditions of prenatal alcoholization. InTopical issues of modern science and education: collection of articles of the International Scientific and Practical Conference: in 2 hours.Penza, Science and Education, 2020: 203-206.
30. Shirpoor A, Gaderi R and Naderi R. Ethanol exposure in prenatal and early postnatal induced cardiac injury in rats: involvement of oxidative stress, Hsp70, ERK 1/2, JNK, and apoptosis in a 3-month follow-up study.*Cell Stress Chaperones*.2019; **24** (5): 917-926. https://doi.org/10.1007/s12192-019-01015-w