Histological analysis of morphostructural changes in bronchial and lung tissues in cattle fetuses during chlamydia infection in Perm Krai and Tyumen Oblast of the Russian Federation

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Abstract. Between 2017 and 2019, 543 cattle (cows’ aborted fetuses, stillborn calves) were studied in Perm Krai and Tyumen Oblast of the Russian Federation for the existence of chlamydia infection. Blood serum samples and fingerprints were analyzed from the animals, and a positive result was registered in 48 animals, which is about 9% of the population. Chlamydia was diagnosed on the basis of clinical signs of disease, passive hemagglutination test and microbiological studies. From 23 aborted and stillborn fetuses, biological material was taken for electron microscopic examination. In order to study pulmonary tissue injuries under the influence of chlamydia, a histological analysis of bronchi and lung aborted and stillborn calves was carried out. All three components of the mother-placenta-fertility system were found to be affected by bovine chlamydia. A characteristic pathological-anatomical sign of chlamydia in fetal death is the formation of common swelling, both tissue and cavity localization. Histological examination of organs and tissues traces changes in the general pathological nature of the developing vascular level, alternative processes, immunopathological reactions, systemic inflammatory changes in histochemical barriers. Morphological changes in the pulmonary tissue of the fetuses of different gestation terms indicate damage to the aero-hematic barrier with the possibility of respiratory disorders in the uterine period.

1. Introduction
Currently, chlamydia infection is of particular relevance, which is largely determined by the spread of chlamydia, the multifaceted interaction of the infectious agent with the cell, the variety of variants of the flow of the infectious process and the polymorphism of clinical manifestations [1]. In livestock farms, chronic infections are a major national economic problem due to the prolonged course and significant damage caused to young animals, mostly breeding animals [2,3].
Studies of diseases of cattle chlamydia were conducted in the period from 2017 to 2019 in farms of the Perm region: OOO Rus, Perm District, Perm Meat Processing Plant and farms in the Tyumen Region: CJSC Uspenskoye, CJSC Sovkhoz Chervishhevsky, Tyumen District, Russian Federation.

Diagnosis for cow chlamydia was based on clinical signs of disease, passive hemagglutination test, microbiological studies (stamp staining of smears) and electron microscopy. Biological material for histological studies of morphological changes was taken from aborted and stillborn fetuses at the age of 8-9 months.

2. Equipment and devices used in studies
The study involved abortion of cattle and stillborn animals. Diagnosis for animal chlamydia was made by passive hemagglutination test and microbiological studies - smear staining - stamp prints. After the animals were killed, the material for the study was taken and fixed in 10% formaldehyde. The next day, pieces of tissue were cut out, then the alcohols of the increasing strength were routed and the material was poured into paraffin. Histological sections were stained with hematoxylin and eosin. Hematoxylin stains cell nuclei and chromatin in blue-violet tones. Eosin stains cytoplasm and some structures (fibers) in pink-red/orange colours - a review technique, and according to Van Gieson to determine the degree of severity of scleroplastic processes. From the ready blocks on the sled microvolume were made slices up to 5 microns thick. The obtained preparations were studied with the study of the Zeiss microscope (Axioskop 40) at magnification of eyepiece x10, with lenses x4, x10.

3. The results of the study and their discussion
It has been established that along with the development of inflammatory processes of the pelvic organs and infertility, chlamydia infection can lead to a complicated course of pregnancy, significantly worsening the condition of the fetus and the newborn. The results of the studies have shown that even if the process is minimal in an adult, a complex of pathological reactions may develop in the placenta tissues. Infection of animals in later terms of pregnancy is accompanied by a delay of intrauterine fetal development, the formation of secondary placental insufficiency, local and generalized infectious diseases. If the fetus is infected shortly before birth, the manifestations of intrauterine infection depend on the stage of the disease. If at the time of birth the disease is in the initial phase, the infection manifests for the first time 3 days of early neonatal period, if the delivery falls on the final stage of the disease, more often there is a syndrome of disadaptation in the early neonatal period, in the future it is possible to chronicle the process, persistent latent infection.

Histological studies of morphostructural changes in bronchial and lung tissues have shown that there is swelling of the laryngeal, tracheal and bronchial mucosa on the respiratory tract. Macroscopic lungs occupy the pleural cavities by about 2/3, pressed against the roots. The pleura is semi-transparent, moist, shiny, it is randomly defined at the level of different sections of the hemorrhage. The section of the lung tissue is reddish brown and homogeneous. The branches of the pulmonary artery contain small amounts of liquid blood. The section also shows the walls of the bronchi with a loose pinkish mucous membrane. In the lumen of the trachea and bronchi, poor, parietal, grayish mucous masses are visible.

Microscopically, pulmonary tissue is in a state of widespread atelectaza, which is also found by other authors conducting research in this direction [4]. The blood circulation disorders are rather significant - a pronounced full blood of the pulmonary artery branches, vessels of bronchial walls, capillaries of interalveolar partitions are shown in figure 1.

Vessel luminescence is narrowed, nuclei of endothelial cells are clearly visible, cell groups are in a state of desquamation into vessel luminescence. The walls of venous vessels are thin. Arterial vessels are thick-walled due to widespread edema, which is transferred to perivascular parts of the lung tissue [5,6]. The volume of fibrous structures, especially elastic ones, has been increased in the arterial walls; the excess of loose fibrous tissue is also determined perivascularly. These changes are typical for morphological immaturity of vascular walls.
Alveolar walls are thickened and numerous small capillary vessels filled with erythrocytes can be traced in them [7]. Swelling is quite common in interstitial zones, and cellular elements of the lymphomacrophage series with admixture of single plasma cells are visible (figure 2).

Figure 1. Immature alveolar and bronchial structures, atelectasis of the lung fetus. Colour with hematoxylin and eosin. х400.

Figure 2. Lymphoid-macrophagal infiltration in alveols with fibrin impurity in fetal lungs. Colour with hematoxylin and eosin. x400.

Thus, due to the full blood of capillaries, pericapillary edema and cellular infiltrates, the interstitial zones are thickened. These morphological changes are a sign of intrauterine infection. Due to the small volume of alveoli, the interlateral layers represented by loose edematous fibrous tissue are clearly visible (figure 3).

The alveoli are sticking together; the gaps are not clearly visible. In case of prematurity or pathological immaturity of lung tissue, the alveoli have a tubular structure (figure 4).

In such cases, they are lined with cubic epithelial cells with an uneven distribution of nuclei [8]. Boca-like cells in the epithelial lining are not traceable. Such morphological immaturity of lung tissue and weak differentiation of cellular elements may have a negative impact on the formation of external respiration function in the postnatal period due to the absence or insufficient functioning of type 2 alveolocytes, the activity of which consists in the development of surface-active substances that give
stability to the alveolus on inhalation and exhalation [9,10]. In places, clearly visible emphysema areas with a rupture of interalveolar partitions are traced (figure 5).

**Figure 3.** Pronounced edema of interannulus connective tissue in the lungs of the fetus. Colour with hematoxylin and eosin. x 200.

**Figure 4.** Tubular alveolar structures of immature lung. Colour with hematoxylin and eosin. x 100.

**Figure 5.** Acute emphysema of the light fetus. Color hematoxylin and eosin.x100.
In the alveolar lumen we can see groups of listened alveolar macrophages, pinkish eosinophilic masses, erythrocytes, lymphocyte groups. Alveolocytes of type 2 have a rounded shape, their cytoplasm is vacuumized, the nuclei can be pushed to the periphery. Separate nuclear-free cells are found. In some cases, single neutrophils are added to the described content of alveoli, which may indicate the beginning of intrauterine pneumonia (figure 6).

Figure 6. Thickening of the interalveolar partitions of the lung. Inflammatory exudate in alveoli lumen. Colour with hematoxylin and eosin. x100.

Bronchial luminescence is narrowed and has a festoon appearance due to the formation of true epithelial papillae. Bronchial epithelium in a state of desquamation, dystrophy, disorganization, nuclei of epithelial cells are large, optically transparent, bubbly-shaped. Lashes of epithelial cells are in a state of agglutination or are not differentiated. The number of glass-shaped cells is reduced. In some places, cells are in the bronchial lumen in the form of small groups with an admixture of mucous masses, lymphocytes, and macrophage cells. In the walls of the bronchi - a pronounced full blood vessels, widespread swelling, excess fiber tissue. The presence of cartilage islets in the walls of small bronchi testifies to the morphological immaturity of the bronchial tree structures.

Changes in the local ”immune status” of pulmonary tissue and bronchial tree are characteristic. Small clusters of mature lymphocytes are visible around large vessels and bronchi, in some places lymphoid follicles containing full-blooded vessels, with an indistinct macrophage reaction at the follicle level. This indicates the intensity of local immunity and the strengthening of the barrier function of lymphoid formations.

Pleura in the form of small fragments of fibrous tissue with swelling, small groups of erythrocytes, lymphocytes, macrophage cells, the presence of single capillary vessels, often full-blooded.

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4. Conclusion
The obtained data of morphological changes testifies to the damage of the aero-hematic barrier with the possibility of respiratory disorders in the off-uterine period of fruits. Here, blood circulation disorders in the form of vessel blood and edema development, excessive presence of fibrous tissue in the main lung structures, damage to functionally active cells of the alveolar wall, presence of cellular elements and edema in the alveolar lumen, changes in the bronchial epithelium, activation of local immune mechanisms are significant. Lungs with such morphological changes are not fully adapted to the extrauterine functioning, which is dangerous in terms of the development of common atelectazes, pneumopathy and pneumonia in a newborn calf.

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