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# ANTIOXIDANT STATUS AND FUNCTIONAL CONDITION OF RESPIRATORY SYSTEM OF NEWBORN CALVES WITH INTRAUTERINE GROWTH RETARDATION

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## Abstract

Intrauterine fetal and embryo growth retardation (IUGR), defined as a discrepancy of embryo forming and fetus size and their gestation terms, is a common pathology among farm animals. Respiratory dysfunctions in newborns with IUGR are among the factors leading to animal death from birth to weaning. The immature antioxidant defense system (AOS) of newborns with IUGR predisposes to oxidative stress progression and associated pathologies. We show in this paper the lack of enzymatic and non-enzymatic links of antioxidant protection, an increased concentration of malonic dialdehyde in blood and in exhaled air, and higher expiration of enzymes of different subcellular localization, i.e. alanine aminotransferase, γ-glutamyl transferase, aspartate aminotransferase, indicating damage to the respiratory tract cells. These data contribute to elucidating mechanisms of respiratory dysfunctions as influenced by IUGR. A comparative study of AOS indicators, functional state of respiratory organs of newborn calves and the respiratory disease progression in the neonatal period was carried out at a large dairy complex (Agrotech-Garant Nashchekino Co. Ltd, Anninsky Region, Voronezh Province) in 2013. A total of 53 red-motley calves were examined, including 28 calves with IUGR in history and 25 ones whose mothers had physiological course of pregnancy (control group). In 24 hours after the calves' birth, switch tail hair samples, blood and exhaled breath condensate (EBC) were collected for biochemical studies, the heart rate (HR) and frequency of respiratory rate (RR) per minute, the ratio of HR/RR (Hildebrandt index), tidal volume (TV) and respiratory minute volume (RMV), the volume of EBC produced per minute (V1) and from 100 liter of exhaled air (V2) were determined. The hair concentrations of iron, copper, zinc, manganese, selenium and cobalt were determined by atomic absorption spectrophotometry (Shimadzu AA6300, Japan); the activity of catalase, selenium-dependent glutathione peroxidase (GPO), superoxide dismutase (SOD) in blood, the blood concentration of malonic dialdehyde (MDA), the serum (plasma) content of vitamin A, α-tocopherol, L-ascorbic acid and total antioxidant activity (AOA) were studied spectrophotometrically (Shimadzu UV-1700, Japan). The MDA concentration (Shimadzu UV-1700, Japan), intensity of iron-induced chemiluminescence (BHL-07, Russia), the activity of alanine aminotransferase (ALAT), γ-glutamyltransferase (GGT) and aspartate aminotransferase (ASAT) (Hitachi-902, Japan) were examined in EBC of calves. In the calves with IUGR, as compared to the control group, blood catalase activity reduced by 14.4 % ($P < 0.001$), GPO by 14.0 % ($P < 0.001$) and SOD by 33.8 % ($P < 0.001$), blood serum content of vitamin A decreased by 36.7 % ($P < 0.05$) and α-tocopherol by 38.3 % ($P < 0.001$), while blood plasma AOA was higher by 18.6 % ($P < 0.01$), hair concentration of copper decreased by 28.3 % ($P < 0.001$), zinc by 10.7 % ($P < 0.001$), manganese by 9.4 % ($P < 0.001$), selenium by 26.4 % ($P < 0.001$) and cobalt by 36.8 % ($P < 0.001$), the MDA level in blood and EBC increased by 26.8 % ($P < 0.001$) and 119.5 % ($P < 0.001$), respectively, also, intensity of chemiluminescence outbreak Imax and the light sum of chemiluminescence S of EBC were higher by 36.2 % ($P < 0.01$) and 40.6 % ($P < 0.01$), respectively. An increase in the ratio of S/tg2α in EBC of calves with IUGR (by 35.5 % compared to the control group, $P < 0.01$) indicated imbalance of oxidative and antioxidant activity of EBC and oxidative stress progression. Structural and functional damage of respiratory tract under oxidative stress of IUGR calves was accompanied by an increase in ALAT expiration by 105.9 % ($P < 0.001$), GGT by 416.1 % ($P < 0.001$), ASAT by

62.5 % ($P < 0.001$), and respiratory moisture release (V2) by 67.3 % ($P < 0.001$) compared to the control group. An increase in Hildebrandt index of calves with IUGR (by 7.9 % compared to the control group, $P < 0.05$) indicates the autonomic regulation disorder and the cardiorespiratory functional system overstrain. A statistically significant relationship was found between the risk of bronchopneumonia development and the S/tg2α ratio which reflects the balance of EBC oxidative and antioxidant activity ($r_{\tau\text{-K}} = +0.58$, $P < 0.01$), and also the blood activity of catalase ($r_{\tau\text{-K}} = -0.68$, $P < 0.01$), GPO ($r_{\tau\text{-K}} = -0.36$, $P < 0.05$) and SOD ($r r_{\tau\text{-K}} = -0.62$, $P < 0.01$).

Keywords: intrauterine fetal and embryo growth retardation, newborn calves, antioxidant defense system, oxidative stress, exhaled breath condensate, respiratory diseases, bronchopneumonia

Intrauterine fetal and embryo growth retardation (IUGR), defined as discrepancy of embryo forming a fetus size and their gestation terms is still a serious problem of animal farming [1-3]. Academic interest toward IUGR is caused not only by the fact that it is a common syndrome affecting pregnant animals [1, 4], but also by its negative impact on postnatal growth and health of the offspring [1, 5-7]. Postnatal hypoglycemia, hypoxemia [1, 8], hypersensitivity to hypothermia resulting from dysfunctional thermoregulatory functions and low energy stores of the body [8-10] are common in newborns with IUGR. Respiratory dysfunctions in newborns with IUGR are among the factors leading to animal death in the period between the birth and weaning [1, 5, 11].

Although clinical condition of such animals may appear normal, their internal organs are morphologically and functionally immature [12-14]. According to the study on swine [15], immature antioxidant defense system in newborns with IUGR predisposes to oxidative stress progression and postnatal metabolic disorders.

We have first conducted comparative studies of the antioxidant protection system indicators and functional state of respiratory organs of newborn calves during physiological pregnancy and the IUGR. Newborn calves with IUGR appeared to have deficient enzymatic and non-enzymatic links of the antioxidant protection system, as well as increased concentration of malonic dialdehyde in blood and in exhaled breath condensate, and higher expiration of enzymes of various sub-cellular localization (alanine aminotransferase, γ-glutamyltransferase, aspartate aminotransferase), indicative of damage to respiratory tract cells in conditions of oxidative stress.

The study aims to get an insight into antioxidant status indicators and functional state of respiratory system of the newborn calves with intrauterine growth retardation and the impact of these dysfunctions on the respiratory disease progression over the neonatal period.

*Techniques.* A total of 53 red-motley calves were examined, including 28 calves with IUGR in history and 25 calves whose mothers had physiological course of pregnancy (control group); the studies were carried out in 2013 at Agrotech-Garant Nashchekino Co. Ltd. (Anninsky Region, Voronezh Province). The cows were diagnosed for IUGR by way of transrectal palpation and ultrasonic scanning with the help of Easi-Scan-3 (BCF Technology Ltd., U.K.) with 4.5-8.5 MHz linear array probe. Embryo and fetus growth retardation criteria included the crown-rump length and body diameter on day 38-day 45 following insemination and conception — less than 16 mm and 9 mm respectively; on day 60-day 65 — less than 45 mm and 16 mm respectively; on day 110-day 115 — horn-uterus diameter less than 15 cm and placentoma less than 17 mm [2, 4].

Biochemical studies were conducted on samples of hair, blood and exhaled breath condensate (EBC) collected from calves 24 hours after their birth. Hair samples were taken from switch tails, blood samples were drawn from the jugular vein using commercial vacuum blood sampling systems (with EDTA as anticoagulant). EBC samples were collected from calves using our proprietary de-

vice [16]. Blood serum samples were obtained through centrifugal process that took 10 minutes at room temperature and without addition of anticoagulant (4000 rpm, UC-1612, ULAB, China). Immediately upon collection, the serum and EBC samples would be frozen and stored in liquid nitrogen at –196 °C until biochemical studies.

Hair samples were prepared by wet ashing technique and the content of minor elements (selenium, copper, zinc, iron, cobalt and manganese) was determined using the atomic absorption spectrophotometry (Shimadzu AA6300, Japan).

Assessment of the state of enzymatic link of the antioxidant protection system in blood involved measurement of catalase activity (EC 1.11.1.6) and selenium-dependent glutathione peroxidase (GPO, EC 1.11.1.9) using the relevant methods described by M.I. Retsky et al. (17); and activity of superoxide dismutase (SOD, EC 1.15.1.1) in blood was measured by the rate of inhibition of adrenaline autoxidation [18]. The state of non-enzymatic link of the antioxidant protection system was measured by the content of vitamin A [19], α-tocopherol [17], L-ascorbic acid [20] in blood serum and by total antioxidant activity (AOA) of blood serum [21]. Concentration of malonic dialdehyde (MDA) in blood and EBC was measured spectrophotometrically (Shimadzu UV-1700, Japan) by reaction with thiobarbituric acid [17].

Intensity of iron-induced chemiluminescence in EBC was studied with the help of biochemiluminometer BHL-07 (Medozones, Ltd., Russia) by the methods described by Y.G. Voronkova et al. [22], with slight modifications. EBC (0.2 ml), 0.4 ml of 0.02 M potassium-phosphate buffer (pH 7.5) and 0.4 ml of 0.01 M iron (II) sulfate solution were added into the measuring cell successively. The cell with the resulting blend was then placed in the measuring slot of the device, quickly adding 0.2 ml of 2% of hydrogen peroxide, and moving the cell into the measuring position. Free radical process was registered for 30 seconds, chemiluminescence was measured on the basis of the kinetic curve by the following indicators: maximum intensity of the outbreak (that characterizes the intensity of free radical oxidation) — Imax, mV; chemiluminescence light sum (representing the oxidative activity) — S, mV × sec; kinetic curve slope ratio against the time axis (characterizing the antioxidant activity) — tg2α. To determine the balance between oxidative and antioxidant activity, we calculated the ratio of S/tg2α [23]. Activity of alanine aminotransferase (ALAT), γ-glutamyl transferase (GGT) and aspartate aminotransferase (ASAT) in EBC were studied on biochemical analyzer Hitachi-902 (Roche Diagnostics, Japan).

The heart rate (HR) and frequency of respiratory rate (RR) per minute were measured in calves; external respiration functions (tidal volume and respiratory minute volume) were studied with the help of SSP lung tester (KPO Medapparatura, Ukraine) and a mask with valves; Hildebrandt index was calculated as the HR/RR ratio. Animals were under continuous clinical supervision from their birth to day 30 inclusively: their condition in the course of respiratory disease was rated under WI system [24], recording the time of first clinical indications and the height of bronchitis, morbidity (0 — no pathology, 1 — mild, 2 — moderately severe and 3 — severe disease), complication in the form of bronchial pneumonia.

Statistical data processing was done in Statistica 8.0 (StatSoft, Inc., USA) and IBM SPSS Statistics 20.0 (IBM Corp., USA) software. The results represent mean arithmetic and standard deviation ($M \pm SD$), minimum (min), maximum (max) and median values (*Me*). The significance of difference between median values of the samples was measured by means of nonparametric Wilcoxon test. Correlations between the values were identified by means of Spearman rank

correlation coefficient ($r_S$) and Kendall's τ ($r_{\tau\text{-K}}$). Zero hypothesis was disregarded in all statistical processing methods at $p < 0.05$.

*Results.* According to the study of blood of claves with IUGR, activity of catalase was found to be down by 14.4% ($p < 0.001$), GPO by 14,0% ($p < 0.001$) and SOD by 33.8% ($p < 0.001$); vitamin A content in blood serum was down by 36.7% ($p < 0.05$) and α-tocopherol down by 38.3% ($p < 0.001$); blood serum AOA was up by 18.6% ($p < 0.01$) compared to control group (Table 1). No statistically significant differences in L-ascorbic acid concentrations in blood serum of various groups of calves were observed.

**1. Indicators of antioxidant system in blood (serum) of newborn red-motley calves — normal and with intrauterine fetal growth retardation** (Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| Catalase, μmol $H_2O_2$/(l · min) | 26.7±1.4 | 23.6-28.7 | 26.9*** |
| | 31.2±3.0 | 27.3-35.3 | 30.6 |
| GPO, μmol GSH/(l · min) | 6.84±0.70 | 5.56-7.84 | 6.90*** |
| | 7.95±0.71 | 6.71-8.72 | 8.31 |
| SOD, conventional units | 0.53±0.07 | 0.43-0.65 | 0.53*** |
| | 0.80±0.09 | 0.62-0.92 | 0.81 |
| Vitamin A, μmol/l | 0.57±0.22 | 0.27-0.87 | 0.54* |
| | 0.90±0.35 | 0.60-1.60 | 0.73 |
| A-tocopherol, μmol/l | 5.0±2.2 | 2.8-8.4 | 4.0*** |
| | 8.1±1.6 | 5.5-9.9 | 8.7 |
| L-ascorbic acid, μmol/l | 17.8±6.8 | 10.9-30.6 | 17.3 |
| | 23.4±5.1 | 17.3-36.6 | 22.8 |
| AOA,% | 42.5±7.7 | 27.9-48.0 | 44.4** |
| | 52.2±4.2 | 47.6-61.0 | 51.7 |

N o t e. GSH — reduced glutathione, GPO — glutathione peroxidase, SOD — superoxide dismutase, AOA — total antioxidant activity in blood serum. Above the line — group of calves with intrauterine growth retardation ($n = 28$), below the line — group of calves whose mothers had physiological course of pregnancy ($n = 25$).
*, **, *** Differences between the groups are statistically significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively.

Concentration of virtually every microelement (excluding iron) covered by this study was found to be down in the hair of newborn calves with IUGR: copper by 28.3% ($p < 0.001$), zinc by 10.7% ($p < 0.001$), manganese by 9.4% ($p < 0.001$), selenium by 26.4% ($p < 0.001$) and cobalt by 36.8% ($p < 0.001$) compared to control group (Table 2).

Correlation analysis detected statistically significant correlation between copper content in calves' hair and SOD activity in blood ($r_S = +0.55$ at $p < 0.05$), as well as selenium content in hair and GPO activity in blood ($r_S = +0.84$ at $p < 0.01$).

**2. Microelements content in tail hair of newborn red-motley calves — normal and with intrauterine fetal growth retardation** (Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013)

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| Iron, mg/kg | 31.7±8.6 | 20.3-47.0 | 30.4 |
| | 37.5±13.0 | 20.3-65.0 | 34.4 |
| Copper, mg/kg | 6.52±1.30 | 3.15-8.0 | 7.01* |
| | 9.09±1.01 | 7.4-10.6 | 9.35 |
| Zinc, mg/kg | 105.6±14.2 | 88.7-127.3 | 98.8* |
| | 118.2±15.8 | 91.3-138.1 | 121.6 |
| Manganese, mg/kg | 8.55±0.27 | 8.11-9.01 | 8.50* |
| | 9.44±1.22 | 8.11-12.8 | 9.19 |
| Selenium, μg/kg | 345.0±67.4 | 261.0-447.0 | 317.5* |
| | 468.5±69.4 | 398.0-595.0 | 457.5 |
| Cobalt, μg/kg | 42.5±10.1 | 25.4-56.0 | 42.9* |
| | 67.3±15.2 | 51.2-96.8 | 62.7 |

N o t e. Above the line — group of calves with intrauterine growth retardation ($n = 28$), under the line — group of calves whose mothers had physiological course of pregnancy ($n = 25$).
* Differences between the groups are statistically significant at $p < 0.001$.

Increase of MDA concentration in blood and EBC of calves with IUGR

by 26.8% ($p < 0.001$) and 119.5% ($p < 0.001$) respectively compared to control group (Fig. 1) was indicative of the increasing systemic and local (lungs) intensity of lipids peroxidation associated with functional deficiency of enzymatic and non-enzymatic links of the antioxidant protection system.

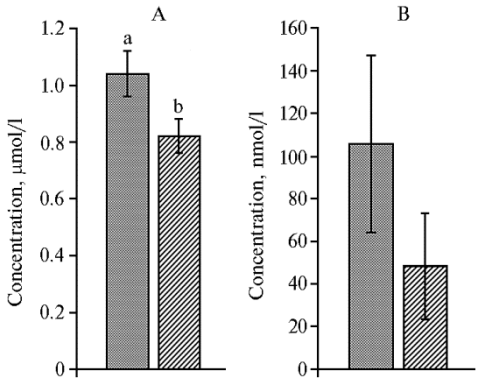


**Fig. 1. Malonic dialdehyde** (MDA) **content in blood** (A) **and in exhaled breath condensate** (B) **of newborn red-motley calves with intrauterine fetal growth retardation** (a) **and physiological course of pregnancy of cows** (b) (*M*±SD, Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013).

Studies of the iron-induced chemiluminescence EBC (Table 3) in newborn calves with IUGR registered the chemiluminescence outbreak Imax and the light sum S EBC by 36.2% ($p < 0.01$) and 40.6% ($p < 0.01$) respectively compared to control group, which was indicative of the increased oxidative activity in EBC. The Imax and S/tg2α indicators correlated with MDA and EBC concentrations: $r_S = +0.78$ ($p < 0.01$) and $r_S = +0.48$ ($p < 0.01$) respectively. No significant differences between the groups of calves in terms of tg2α have been detected. The increasing ratio S/tg2α in EBC of calves with IUGR (by 35.5% compared to control group, $p < 0.01$) is indicative of imbalanced oxidative and antioxidant activity of EBC the development of oxidative stress. Statistically significant correlations were found between S/tg2α in EBC and the activity of antioxidant enzymes in blood, i.e. catalase ($r_S = -0.54$ at $p < 0.01$), GPO ($r_S = -0.49$ at $p < 0.01$) and SOD ($r_S = -0.85$ at $p < 0.01$).

**3. Indicators of iron-induced chemiluminescence in exhaled breath condensate of newborn red-motley calves — normal and with intrauterine fetal growth retardation** (Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013)

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| S, mV × sec | 417.3±37.6 | 374.0-472.0 | 411.5* |
| | 296.9±49.1 | 248.0-422.0 | 282.0 |
| Imax, mV | 65.8±4.7 | 61.0-73.0 | 64.5* |
| | 48.3±10.0 | 40.0-73.0 | 43.0 |
| tg2α | 20.3±1.7 | 18.0-22.5 | 20.3 |
| | 19.5±2.4 | 18.0-25.5 | 18.0 |
| S/tg2α | 20.6±1.1 | 19.2-22.1 | 20.6* |
| | 15.2±1.0 | 13.4-16.5 | 15.7 |

N o t e. Above the line — group of calves with intrauterine growth retardation ($n = 28$), under the line — group of calves whose mothers had physiological course of pregnancy ($n = 25$).
* Differences between the groups are statistically significant at $p < 0.01$.

In EBC of calves IUGR, there was an increase in activity of ALAT by 105.9% ($p < 0.001$), GGT by 416.1% ($p < 0.001$), and ASAT by 62.5% ($p < 0.001$) compared to control group (Fig. 2). Increased activity of enzymes of various subcellular localization (cytoplasmic ALAT, membrane-bound GGT, mitochondrial ASAT) in EBC of calves with intrauterine growth retardation reflects the extent of structural and functional damage to the respiratory tract cells (from disruption of biomembrane permeability to cytolysis) and is caused by the occurrence of these components in epithelial lining fluid [26, 27]. We have established statistically significant correlation between the GGT activity in EBC and the intensity of free radical oxidation Imax ($r_S = +0.51$ at $p < 0.01$), ratio S/tg2α reflecting the balance between the oxidative and anti-

oxidant activity EBC ($r_S$ = +0.74 at p < 0.01), as well as GPO ($r_S$ = –0.55 at p < 0.01) and SOD ($r_S$ = –0.53 at p < 0.01) activity in blood. Similar correlations have been found between the ASAT activity in EBC and the S/tg2α ratio ($r_S$ = +0.41 at p < 0.05), GPO ($r_S$ = –0.52 at p < 0.01) and SOD ($r_S$ = –0.38 at p < 0.05) activity in blood, and between the ALAT activity in EBC and the value of antioxidant activity EBC tg2α ($r_S$ = –0.39 at p < 0.05).

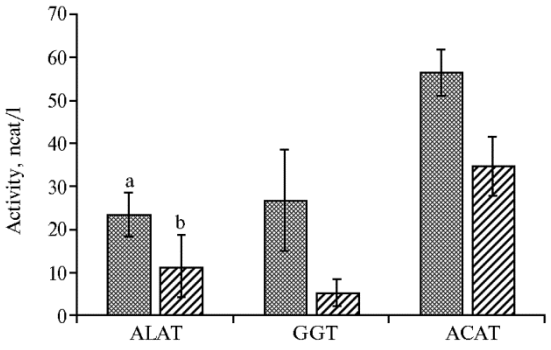


**Fig. 2. Enzyme activity in the breath condensate exhaled by newborn red-motley calves:** a — with intrauterine fetal growth retardation, b — physiological course of pregnancy; ALAT — alanine aminotransferase, GGT — γ-glutamyl transferase, ASAT — aspartate aminotransferasea (*M*±SD, Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013).

Increased intensity of respiratory moisture was detected in calves with IUGR against the background of the respiratory minute volume decrease by 34.0% (p < 0.001) and the breathing capacity by 20.9% (p < 0.001): the amount of EBC produced over the period of 1 minute out of 100 liters of the exhaled air would increase compared to control group by 28.6% (p < 0.01) и 67.3% (p < 0.001) respectively (Table 4). We have detected inverse relationship between the amount of condensate produced out of 100 liters of the exhaled air and the depth of breathing ($r_S$ = –0.61 at p < 0.01). The increase in Hildebrandt index in calves with IUGR (by 7.9% against the control group, p < 0.05) was indicative of the autonomic imbalance and cardio-respiratory system overload [25].

**4. Respiratory and moisture producing function of the lungs of red-motley calves — normal and with intrauterine fetal growth retardation** (Agrotech-Garant Nashchekino Co. Ltd., Anninsky Region, Voronezh Province, 2013)

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| RR, $min^{-1}$ | 46.3±8.1 | 23.6-28.7 | 44.0* |
| | 57.7±15.7 | 27.3-35.3 | 52.0 |
| RMV, l | 9.5±2.2 | 7.4-12.5 | 8.7** |
| | 14.4±2.8 | 10.6-19.1 | 13.4 |
| TV, ml | 210.7±55.8 | 153-284 | 195.0** |
| | 266.3±88.3 | 156-445 | 254.0 |
| V1, ml | 0.09±0.03 | 0.06-0.12 | 0.10* |
| | 0.07±0.02 | 0.05-0.09 | 0.07 |
| V2, ml | 0.92±0.31 | 0.59-1.42 | 0.84** |
| | 0.55±0.09 | 0.39-0.72 | 0.53 |

N o t e. RR — respiratory rate, RMV — respiratory minute volume, TV — tidal volume; V1 and V2 — volume of exhaled breath condensate produced over the period of 1 minute out of 100 liters of exhaled air. Above the line — group of calves with intrauterine growth retardation ($n$ = 28), below the line — group of calves whose mothers had physiological course of pregnancy ($n$ = 25).
*, ** Difference between the groups is statistically significant at p < 0.01 and p < 0.001 respectively.

Respiratory diseases were registered in 48.0% of calves whose mothers had physiological course of pregnancy and in 100% calves with IUGR; severe bronchitis developing into bronchial pneumonia occurred in 12.0% and 85.7% of animals respectively. We have identified statistically significant relationship between the potential development of bronchial pneumonia in calves and the S/tg2α ratio that reflects the balance of pro- and antioxidant activity of EBC ($r_{\tau\text{-}K}$ = +0.58 at p < 0.01), and the activity of catalase in blood ($r_{\tau\text{-}K}$ = –0.68 at p < 0.01), GPO ($r_{\tau\text{-}K}$ = –0.36 at p < 0.05) and SOD ($r_{\tau\text{-}K}$ = –0.62 at p < 0.01).

Rapid evolution of fetus from intrauterine hypoxic environment to normoxia and pulmonary respiration at the time of birth is accompanied by con-

siderable stress on every functional system of the body [28-30]. Beginning of pulmonary respiration is associated with the increased generation of reactive oxygen intermediates (ROI) and oxidative stress development [28, 31]. In turn, the oxidative stress is associated with a number of pathological conditions in the newborn animals — cardiovascular and pulmonary disorders, lactic acidosis, reduced absorption and passive passage of nutrients and immunoglobulins in the intestinal tract [15, 28, 31]. Excess ROI generation is compensated by the adaptive changes in the antioxidant protection system [32-35], mostly enzymatic part thereof [31, 32, 35].

As the cattle tail switch hair begins to grow on month 7 of pregnancy [36, 37], concentration of chemicals in the hair samples collected from this part of the body (entire length of the hair stem) on day 1 after the birth may be considered as the integral indicator of minerals supply in the calves over the last 3 months of intrauterine development. In the period of 10%-15% of the term preceding the completion of gestation, the activity of antioxidant enzymes in fetal tissues is known to increase by 150%-200% [33, 38]. According to the findings of our studies, unlike the calves whose mothers had physiological course of pregnancy, calves with intrauterine growth retardation had copper concentration in their tail hair smaller by 28.3% ($p < 0.001$), zinc by 10.7% ($p < 0.001$), manganese by 9.4% ($p < 0.001$), selenium by 26.4% ($p < 0.001$), and cobalt by 36.8% ($p < 0.001$). According to the obtained data, in the event of IUGR, the enzymatic part of the antioxidant protection system in fetus develops in conditions of deficient amount of copper, zinc, manganese, selenium and cobalt. In calves with IUGR, the catalase activity in blood was down by 14.4% ($p < 0.001$), GPO by 14.0% ($p < 0.001$), and SOD by 33.8% ($p < 0.001$) compared to the control group. Strong relationship between the deficient microelements and functional disorder of enzymatic part of the antioxidant protection system in newborn calves was confirmed by correlation between copper concentration in the hair and the SOD activity in blood ($r_S = +0.55$ at $p < 0.05$), and selenium concentration in the hair and GPO activity in blood ($r_S = +0.84$ at $P < 0.01$). D. Shukla et al. [39] prove the importance of cobalt in antioxidant protection of the lungs, so deficit of cobalt in calves with IUGR takes on particular significance.

Considerable drop in concentration of vitamin A in blood serum, α-tocopherol and overall antioxidant activity of blood serum has been detected in calves with IUGR (see Table 1). Oxidative stress development in the lungs, however, appeared to be mostly tied to deficient enzymatic part of the antioxidant protection system, which has been confirmed by statistically significant relationship between S/tg2α in EBC and the catalase activity ($r_S = –0.54$ at $p < 0.01$), GPO ($r_S = –0.49$ at $p < 0.01$) и SOD ($r_S = –0.85$ at $p < 0.01$) in blood, as well as by the findings of the studies conducted by other authors [33, 40, 41].

In calves with IUGR, we have observed the increase in MDA concentration in blood and EBC (by 26.8% at $p < 0.001$ and 119.5% at $p < 0.001$ respectively, compared to control group), indicative of the increasing systemic and local (lungs) intensity of peroxidation of lipids against the deficient enzymatic and non-enzymatic links of the antioxidant protection system. Our data correlate with the findings of Z. Hracsko et al. [42], which demonstrated significant increase in blood MDA against the decreased activity of catalase, GPO, SOD and the reduced glutathione concentration in the newborns with IUGR compared to children born by mothers who had physiological course of pregnancy.

Structural and functional damage to respiratory tract cells in conditions of oxidative stress in calves with IUGR was accompanied by the increased exhalation of enzymes of various subcellular localization (cytoplasmic ALAT, membrane-bound GGT, mitochondrial ASAT) and intensified release of respiratory

moisture. According to prior studies [29], the increase of respiratory moisture release occurs in newborn calves in the event of hypoxia and acidosis and is caused by metabolic and respiratory adaptation disorder. The increased Hildebrandt index in calves with IUGR (by 7.9% compared to control group, $p < 0.05$) is indicative of the autonomic regulation disorder and cardiorespiratory functional system overstrain [25]. A statistically significant relationship was found between the risk of development of bronchial pneumonia in calves and the S/tg2α ratio which reflects the balance of EBC oxidative and antioxidant activity ($r_{\tau\text{-K}} = +0.58$ at $p < 0.01$), and also the blood activity of catalase ($r_{\tau\text{-K}} = -0.68$ at $p < 0.01$), GPO ($r_{\tau\text{-K}} = -0.36$ at $p < 0.05$) and SOD ($r_{\tau\text{-K}} = -0.62$ at $p < 0.01$). Severe course of bronchitis evolving into bronchial pneumonia in calves with IUGR within neonatal period was registered 7.14 times more frequently ($p < 0.001$) than in calves born by mothers with physiological course of pregnancy.

Therefore, according to the findings of this study, the lack of enzymatic and non-enzymatic links of the antioxidant protection in newborn calves with intrauterine growth retardation causes the oxidative stress, accumulation of toxic products of peroxidation of lipids in blood and epithelial lining fluid, structural and functional damage to respiratory tract cells, and considerably increases the risk of bronchial pneumonia in neonatal period.

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