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# MULTIPLE EFFECTS OF PREECLAMPSIA IN COWS ON POSTNATAL GROWTH AND HEALTH OF OFFSPRING

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## Abstract

Scientific interest in such pathologies of pregnancy as functional deficiency of the fetoplacental system, intrauterine growth retardation of embryo and fetus, and preeclampsia (gestosis), is caused not only by their wide spread among productive animals, but also by negative impact on postnatal growth and the health of offspring. The influence of a preeclampsia on incidence of newborn calves of anemia, an omphalitis, inflammatory diseases of gastrointestinal and respiratory tracts and also intensity of growth in the first two months of life was studied on red-motley cows in 2016 in the conditions of a large dairy complex (Voronezhpishcheprodukt Co Ltd, Novousmansky Rregion, Voronezh Province). Total of 45 cows (*Bos taurus taurus*) with a gestation period of 248-255 days were examined, including 31 with clinical signs of preeclampsia and 14 with the physiological course of pregnancy, and calves obtained from them ($n$ = 45). The cows were assessed for clinical signs of preeclampsia (pathological swelling of mammary gland, ventral abdominal wall, dewlap, pelvic extremities, arterial hypertension and proteinuria), the blood concentrations of progesterone, estradiol and dehydroepiandrosterone sulfate (DHEA-S) were determined, the endogenous intoxication features were examined, i.e. concentration of medium size peptides (MSP), effective (ECA) and total (TCA) serum albumin concentration, the toxicity index TI = (TCA/ECA) – 1 and the intoxication coefficient IC = (MSP/ECA) × 1000 were calculated. Within 1-3 hours after birth, the diameter of the calves' navel was measured; an increase in the diameter of the navel of more than 18.0 mm was considered as a retrospective indicator of the fetoplacental blood circulation disorder. On day 1, day 30 and day 60 the calves' bodyweight was measured; the absolute, relative and average daily weight gain for the first and second months of life was calculated. During the first 60 days of life, the calves underwent constant clinical observation, the incidence of anemia, an omphalitis, gastroenteritis, bronchitis, bronchopneumonia and severity of disease course were considered. Frequency of anemia cases in calves from mother cows with preeclampsia was 1.80-fold, of omphalitis 9.40-fold, of gastroenteritis 1.80-fold, of bronchitis 1.75-fold, and of bronchopneumonia 2.71-fild as compared to animals from cows with the physiological course of pregnancy. For the first month of life, an average daily bodyweight gain of the calves received from the cows suffered from a preeclampsia was 46.2 % ($p < 0.01$) less than that of offspring of healthy cows. We revealed significant individual differences in the protein concentration in urine, endogenous intoxication parameters (ECA/TCA, MSP, IC) and the blood content of steroid hormones (progesterone, estradiol, DHEA-S) among the cows with the symptoms of preeclampsia. An inverse relationship was established between the rate of intoxication in cows with preeclampsia and the average daily bodyweight gain in their calves during the first month ($r_S$ = –0.79, $p < 0.01$) and the second ($r_S$ = –0.58, $p < 0.01$) month of life. As proteinuria in cows increases, the probability of omphalitis progression ($r_{\tau\text{-}K}$ = +0.32, $p < 0.05$) and anemia ($r_{\tau\text{-}K}$ = +0.33, $p < 0.05$) of calves increases too. Statistically significant links are found between accumulation of MMP in blood of cows suffering from preeclampsia, and probability of omphalitis ($r_{\tau\text{-}K}$ = +0.36, $p < 0.01$) and bronchopneumonia ($r_{\tau\text{-}K}$ = +0.35, $p < 0.05$) progression in their offspring and also between coefficient of intoxication and probability of progression of these diseases (with τ-Kendall's coefficients +0.35, +0.35 and +0.38 respectively at $p < 0.05$). The functional disorder of fetoplacental system is associated with progression of omphalitis ($r_{\tau\text{-}K}$ = +0.33, $p < 0.05$), gastroenteritis ($r_{\tau\text{-}K}$ = +0.49, $p < 0.01$) and also early (within the first week of life) manifestation of bronchitis ($r_{\tau\text{-}K}$ = +0.48, $p < 0.01$) in calves, and fetoplacental blood circulation dis-

order is associated with gastroenteritis ($r_{\tau\text{-}K}$ = +0.77, p < 0.01) and bronchopneumonia ($r_{\tau\text{-}K}$ = +0.75, p < 0.01). ROC-analysis showed that the factor of intoxication and the content of MSP in the blood serum of cows suffering from preeclampsia may serve as predictors of omphalitis and bronchopneumonia progression in newborn calves. Blood concentration of estradiol and the ratio of progesterone/estradiol in mother cows are valuable indicators to predict a severe course of gastroenteritis and anemia in their calves. High specificity (94.7 %) for predicting anemia progression for newborn calves is detected for proteinuria of more than 2.0 g/l.

Keywords: preeclampsia, pregnancy, *Bos taurus taurus*, cows, calves, arterial hypertension, proteinuria, anemia, omphalitis, gastroenteritis, respiratory diseases, average daily bodyweight gain, ROC-analysis

Preeclampsia (gestosis) is a pathology of pregnant cows and heifers that manifests as multiple-organ functional failure, systemic endotheliosis, disordered uterine-placental and fetus-placental circulation, endogenous intoxication, and disintegration of the mother-fetus hormonal status [1-4]. According to Kolchina [4], preeclampsia occurs in 42.0 to 69.4% of all cows and heifers aged 32 to 36 weeks.

Despite considerable interest in the problem, the pathogenesis of preeclampsia in cows remains understudied [1, 4, 5]. Health researchers believe [6-8] that preeclampsia is a result of maternal exposure to a number of neurogenic, hormonal, immunologic, placental, and genetic factors during pregnancy. The disease has two stages of progression [7]. Stage One includes disordered trophoblast invasion and remodeled spiral arteries in the endometrium, vascular spasm, reduced uterine-placental perfusion [6]. Stage Two includes systemic inflammatory response and multiple-organ failure [6, 8]. Numerous attempts to reproduce preeclampsia experimentally in lab animals have failed [9]. When ligating the uterine vessels, placental ischemia and fetal growth delays were identified in pregnant rats, rabbits, and monkeys; however, changes in the rheological and coagulation properties of blood, microcirculation disorders, and arterial hypertension, all of which are characteristic of preeclampsia, did not develop [9].

Two groups of causative factors of preeclampsia are identified in dairy cows. The first group is associated with extragenital pathology (hepatopathy and nephropathy) accompanied by endogenous intoxication, metabolic disorders (changed protein and lipid composition of blood, oxidative stress), affected immune status (activation of lysozyme and the complement system), increased aggregation of erythrocytes and thrombocytes, changes in the rheological and coagulation properties of blood, microcirculation disorders and damaged capillary endothelium, especially in the emerging placental tissue [1, 5, 10]. The second group is associated with an endocrine deficiency that adversely affects uterine-placental circulation and causes a diffusion-perfusion failure in the placenta [4, 10]. According to Nezhdanov et al. [10], negative factors may overlap and have a cumulative effect in early preeclampsia.

Classic signs of preeclampsia in cows are arterial hypertension, proteinuria (nephropathy), pathological edema of the mammary gland, ventral abdominal wall, dewlap, and pelvic limbs [1, 4, 10]. These symptoms may manifest individually or in combinations proportionally to the severity of the disease [2, 4, 10]. According to Kolchina [4], edemas are noted in 35.3% of preeclampsia-affected cows and heifers, nephropathy in 4.7%, and polysystemic forms in 40.2%. The disease is characterized by the functional failure of the antioxidant protection system and fetoplacental complex [4].

Morphological studies of maternal and fetal parts of the placenta in preeclampsia-affected cows [4, 11] identified deep vascular changes with elements of congestive hyperemia, hemorrhage, extravasates, and signs of dystrophy. Calves born of such cows have blood-filled umbilical vessels with enlarged lumen and diameter, a partially loosened and interrupting intima endothelium,

blood-filled muscular-shell vessels, scattered myocytes and vacuoles in-between, enlarged and loosened adventitia collagen fibers [12].

These morphofunctional changes in pregnant cows are accompanied by significant changes in metabolism [1, 4] as well as in the structure and functional status of fetal organs and systems [2]. Such changes result in less viable offspring [4, 12, 13] with organ and system maldevelopment at cellular and subcellular levels [14-17], lower natural resistance [2, 12] and lesser adaptive capacities [15, 18, 19].

This paper is the first to demonstrate the diagnostic value of identifying the markers of endogenous intoxication, nephropathy, disordered fetoplacental circulation and endocrine function in preeclampsia-affected cows so as to predict neonatal diseases in their offspring. The researchers have found a correlation between the severity of endogenous intoxication in preeclampsia-affected cows and the daily average weight gain of their calves over the first and the second months of life; another correlation is between intoxication and the probability of omphalitis and bronchopneumonia. Functional failure of the fetoplacental system in cows is shown to be related to omphalitis, gastroenteritis, and early (Week 1) manifestation of bronchitis in calves, while malfunctioning fetoplacental circulation is shown to correlate with gastroenteritis and bronchopneumonia in the offspring. It has been found that a higher concentration of protein in mother cows' urine is associated with a greater likelihood of anemia in newborn calves.

The goal was to study how morphofunctional disorders in preeclampsia-affected cows could affect newborn calves' susceptibility to anemia, omphalitis, inflammatory gastrointestinal or respiratory tract diseases, as well as their growth rate in the first two months of life.

*Techniques.* The research was carried out in 2016 during the winter housing season at OOO Voronezhpishcheprodukt facilities, Novusmansky District, Voronezh Province. The research team examined a total of 45 *Bos taurus taurus* red-motley cows with a gestation age of 248 to 255 days, including 31 cows with clinical manifestations of preeclampsia (Group I) and 14 animals with a normal pregnancy (Group II), as well as their calves ($n$ = 45). Over the previous lactation, the cows had had a milk yield of 6,278 to 9,796 kg with a fat content of 3.54 to 3.96%.

Clinical tests evaluated habitus; nutritional status; edemas in mammary glands, ventral abdominal walls, dewlap, and pelvic limbs; rectal temperature; heart rate and respiratory rate (HR and RR, respectively); systolic and diastolic blood pressure (SP and DP). Researchers also considered the course of delivery (normal delivery, assisted delivery and why it was necessary). Each calf's navel diameter was measured at the base within 1 to 3 hours of life. The navel diameter in excess of 18.0 mm was considered a retrospective marker of fetoplacental circulation disorders [12].

Calves were weighed on Days 1, 30, and 60 to calculate absolute, relative, and daily average body weight gain over the first and the second months of life. For the first 60 days, calves were subject to continuous clinical monitoring with the following readings: rectal temperature, HR and RR, condition of visible mucosa, laryngeal/tracheal/intercostal sensitivity to palpation, time and nature of cough, wheezing, and shortness of breath, nasal bleeding and ocular secretions. Pulmonary ventilation (respiratory volume and minute volume) were measured using an SSP spirometer (KPO Medapparatura, Ukraine) and a valve mask. When diagnosing omphalitis, the researchers paid attention to the presence or absence of edemas in the umbilical area, in the lateral or lower abdominal wall; thickened umbilical cord and navel; skin redness at the navel base; umbilical cord stump color and consistency; navel and umbilical ring tenderness; local

temperature increase. The intestinal inflammatory process was indicated by the fecal presence of soluble protein, hemoglobin, leukocytes, and pH imbalance (too acidic, i.e. <7.0, or too alkaline, i.e. >7.5) [20]. In the case of diarrhea, researchers paid attention to the frequency of defecation, the odor, color, and consistency of feces, the skin turgor, the eyeball retraction, the anal sphincter tone, and the abdominal wall sensitivity to palpation. In the case of the respiratory syndrome, calve condition was WI-scored [21] with due account of when the first clinical signs manifested, when bronchitis manifestations peaked, how severe the disease was, and whether bronchopneumonia complications occurred.

For laboratory tests, blood was sampled from cows 30 days before expected calving, or 24 hours after birth from calves. Samples were taken from the jugular vein using a commercial vacuum pump and EDTA as an anticoagulant. Serum was obtained by an anticoagulant-free centrifuge at 4,000 rpm (UC-1612, ULAB, China) at room temperature over 10 minutes. Serum samples were frozen and stored in liquid nitrogen at –196 °C before biochemical testing.

Cow urine samples were collected in sterile polypropylene containers 30 days before expected calving. The urine concentration of protein was estimated (a PocketChem PU-4210 automatic analyzer, Arkrey, Japan).

Hematological tests used a Micros-60 analyzer (Horiba ABX, France); a leukogram was computed by the standard procedure applied to Romanovsky-stained blood smears. The concentration of progesterone, estradiol, and dehydroepiandrosterone sulfate (DHEA-S) in cow serum was determined by solid-phase immunoassay (ELISA) using a Uniplan AIFR-01 analyzer (Zao Pikon, Russia) and commercial test kits by ZAO NVO Immunotech, Russia. Medium-sized peptide (MSP) content of blood serum was determined by the author's methodology using a Shimadzu UV-1700 spectrophotometer (Shimadzu, Japan). Effective and total albumin concentration (EAC, TAC) in the blood serum was measured (a Shimadzu RF-5301 PC spectrofluorophotometer, Shimadzu, Japan) and kits made by the Physico-Chemical Medicine Research Institute, Russia.

Integral endogenous intoxication indices were calculated by the formulas [23]:

$$\text{Toxicity index (TI)} = (\text{TAC}/\text{EAC}) - 1,$$

$$\text{Intoxication coefficient (IC)} = (\text{MMP}/\text{EAC}) \times 1000.$$

Severity was scored as follows: 0 for healthy cows; 1 for mild disease; 2 for moderate disease; 3 for severe disease. ROC analysis per DeLong et al. [24] was used to determine the diagnostic value of identifying the markers of endogenous intoxication, nephropathy, disordered fetoplacental circulation and endocrine function in preeclampsia-affected cows so as to predict neonatal diseases in their offspring (anemia, omphalitis, gastroenteritis, and bronchopneumonia). To that end, the authors analyzed the ROC parameters, in particular, the area under curve (AUC), which characterizes the diagnostic value of an indicator: excellent at 0.9 to 1.0; very good at 0.8 to 0.9; good at 0.7 to 0.8; average at 0.6 to 0.7; unsatisfactory at 0.6 or lower. Other parameters were sensitivity (%), specificity (%), and cut-off points.

Data were processed statistically by Statistica 8.0 (StatSoft Inc., USA) and IBM SPSS Statistics 20.0.0.1 (IBM Corp., USA). Arithmetic means and standard deviations ($M \pm SD$), minima (min), maxima (max), and the median (*Me*) were calculated. Since the distribution of most indicators was not Gaussian as indicated by Shapiro-Wilk W-test, the significance of inter-group difference was found by comparing the medians by the nonparametric Wilcoxon test. Indicator correlation was identified by nonparametric Spearman ($r_S$) and τ-Kendall ($r_{\tau\text{-}K}$) correlation tests. After applying all the methods, the null hypothesis would be rejected at $p < 0.05$.

*Results.* Clinical tests of preeclampsia-affected cows revealed higher heart

rates (96.8±5.5; *Me* = 98.0) and respiratory rates (26.7±4.2; *Me* = 26.0), an increase of 45.2 ($p < 0.01$) and 44.4% ($p < 0.05$) compared to the normal-pregnancy group. Systolic blood pressure was within 120.0 to 152.0 mmHg (*Me* = 138.0 mmHg), diastolic within 92.0 to 112.0 mmHg (*Me* = 96.0 mmHg), which was 30.2 ($p < 0.01$) and 52.4% ($p < 0.01$) higher than in Group II. Pathological edema of ventral abdominal wall, dewlap, and pelvic limbs was noted in 80.6% of cows with arterial hypertension.

Preeclampsia-affected animals had significant interindividual differences in the urine protein concentrations, endogenous intoxication indices (EAC, TAC, MSP, and IC), and in the steroid hormones concentration in the serum, see Table 1. The urine protein concentration was 1.0 to 3.0 g/l (*Me* = 1.0 g/l) in Group I and 0.0 to 0.3 g/l (*Me* = 0.0 g/l) in Group II. Higher proteinuria was associated with greater likelihood of omphalitis ($r_{\tau\text{-}K} = +0.32$ at $p < 0.05$) and anemia ($r_{\tau\text{-}K} = +0.33$ at $p < 0.05$) in the offspring.

No significant difference in the total and effective albumin concentrations or the EAC/TAC ratio was identified between the groups. The concentration of medium-sized peptides (0.55±0.16 cu; *Me* = 0.55) and intoxication coefficient (23.4±7.4 cu; *Me* = 22.5) in preeclampsia-affected cows was above the median values of the normal-pregnancy group, a difference of 71.9 ($p < 0.05$) and 67.9% ($p < 0.05$), respectively; significant intra-sample variation was observed. Correlation analysis identified statistically significant correlation between the accumulation of medium-sized peptides in the serum of Group I cows and the likelihood of omphalitis ($r_{\tau\text{-}K} = +0.36$ at $p < 0.01$) and bronchopneumonia ($r_{\tau\text{-}K} = +0.35$ at $p < 0.05$) in the offspring; the intoxication coefficient, too, correlated with the likelihood of these diseases (τ-Kendall of +0.35 and +0.38, respectively, at $p < 0.05$).

**1. Clinical and laboratory markers of preeclampsia in red-motley cows** (Novousmansky District, Voronezh Province, 2016)

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| RR min | 26.7±4.2 | 26.0-32.0 | 26.0* |
| | 18.9±2.0 | 16.0-22.0 | 18.0 |
| HR min | 96.8±5.5 | 86.0-104.0 | 98.0** |
| | 68.5±5.3 | 60.0-75.0 | 67.5 |
| systolic BP, mmHg | 139.9±10.0 | 120.0-152.0 | 138.0** |
| | 105.2±6.8 | 95.0-112.0 | 106.0 |
| diastolic BP, mmHg | 100.8±7.3 | 92.0-112.0 | 96.0** |
| | 65.4±8.7 | 56.0-78.0 | 63.0 |
| Proteinuria, g/l | 1.7±0.9 | 1.0-3.0 | 1.0*** |
| | 0.1±0.1 | 0.0-0.3 | 0.0 |
| EAC, g/l | 23.1±4.2 | 14.8-30.2 | 23.7 |
| | 26.1±4.6 | 18.3-36.0 | 24.9 |
| TAC, g/l | 37.0±5.0 | 27.5-48.9 | 37.0 |
| | 39.3±4.2 | 33.3-48.3 | 38.1 |
| EAC/TAC, % | 62.2±6.3 | 53.7-74.9 | 60.8 |
| | 66.5±10.2 | 54.7-93.7 | 63.0 |
| MSP, cu | 0.55±0.16 | 0.30-0.78 | 0.55* |
| | 0.35±0.11 | 0.23-0.56 | 0.32 |
| CI | 23.4±7.4 | 11.1-33.9 | 22.5* |
| | 13.5±4.0 | 7.2-19.4 | 13.4 |
| Progesterone, nmol/l | 47.7±13.8 | 21.6-82.4 | 43.6* |
| | 67.3±22.2 | 50.7-110.7 | 52.5 |
| Estradiol, pmol/l | 96.8±53.4 | 32.4-245.1 | 90.4*** |
| | 435.9±114.1 | 245.1-582.9 | 443.1 |
| DHEA-S, μmol/l | 0.31±0.14 | 0.11-0.46 | 0.30* |
| | 0.49±0.23 | 0.14-0.85 | 0.63 |

N o t e. Values above the line are those of preeclampsia-affected cows ($n = 31$); values below the line are those of the normal-pregnancy group ($n = 14$) HR = heart rate; RR = respiratory rate; BP = blood pressure; EAC = effective albumin concentration; TAC = total albumin concentration; MSP = medium-sized peptides; IC = intoxication coefficient; DHEA-S = dehydroepiandrosterone sulfate.
*, **, and *** Inter-group difference is statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

The blood level of progesterone (47.7±13.8 nmol/l; *Me* = 43.6 nmol/l),

estradiol (96.8±53.4 pmol/l; *Me* = 90.4 pmol/L), and DHEA-S (0.31±0.14 μmol/l; *Me* = 0.30 μmol/l) in Group I was 17.0 ($p < 0.05$), 79.6 ($p < 0.001$), and 52.4% ($p < 0.05$) lower than in Group II. A functional failure of the fetoplacental system we identified in 71.0% of preeclampsia-affected cows. In Group I, a functional failure of the fetoplacental system was associated with omphalitis ($r_{\tau\text{-}K} = +0.33$ at $p < 0.05$) and gastroenteritis ($r_{\tau\text{-}K} = +0.49$ at $p < 0.01$), as well as early (Week 1) manifestation of bronchitis ($r_{\tau\text{-}K} = +0.48$ at $p < 0.01$) in the offspring.

In calves born of preeclampsia-affected cows, the navel diameter was 17.0 to 21.00 mm (18.4±1.1 mm; *Me* = 18.0 mm), which was 33.3% ($p < 0.01$) bigger than that of the calves born from normal pregnancy: 13.0 to16.0 mm (13.9±1.1 mm; *Me* = 13.5 mm). In 38.9% of calves, it was > 18.0 mm, a sign of perinatal fetoplacental circulation disorder [7]. The research team identified a statistically significant correlation of fetoplacental circulation disorders in Group I and the likelihood of gastroenteritis ($r_{\tau\text{-}K} = +0.77$ at $p < 0.01$) and bronchopneumonia ($r_{\tau\text{-}K} = +0.75$ at $p < 0.01$) in their offspring. The severity of gastroenteritis and pneumonia did correlate with the navel diameter in the first three hours of life at Spearman rank correlation coefficients of +0.82 and +0.72, respectively, at $p < 0.01$.

Group I calves had 9.40 times the occurrence of omphalitis, 1.80 times the occurrence of bronchitis, 2.71 times the occurrence of bronchopneumonia, and 1.80 times the occurrence of anemia in Group II, see Table 2. In the group of calves born of preeclampsia-affected cows, omphalitis was severe in 38.7% of all cases, anemia (hemoglobin < 70 g/l) was in 9.7% of all cases, with no such complications in the group of normal pregnancy.

**2. Incidence of neonatal diseases in calves born of red-motley cows: normal pregnancy vs preeclampsia** (Novousmansky District, Voronezh Province, 2016)

| Indicator | Normal pregnancy (*n* = 14) | Preeclampsia (*n* = 31) |
|---|---|---|
| Omphalitis, animals (%): | | |
| total | 1 (7.2) | 21 (67.7) |
| severe | 0 (0) | 12 (38.7) |
| Gastroenteritis, animals (%) | | |
| total | 6 (42.9) | 24 (77.4) |
| severe | 4 (28.6) | 20 (64.5) |
| Bronchitis, animals (%) | 8 (57.1) | 31 (100) |
| Bronchopneumonia, animals (%) | 2 (14.3) | 12 (38.7) |
| Anemia, animals (%) | | |
| total | 2 (14.3) | 8 (25.8) |
| severe | 0 (0) | 3 (9.7) |

Omphalitis in calves was largely attributable to endogenous intoxication, endocrine placenta dysfunction, and disordered fetoplacental circulation in the perinatal period. ROC analysis showed that the intoxication coefficient, MSP and estradiol concentration in the serum of preeclampsia-affected cows could be used as neonatal omphalitis predictors. These indices featured a good diagnostic value (AUC = 0.747, 0.753, 0.706), high sensitivity (76.9% for all indicators), and specificity (52.9; 70.6, and 88.2%). Omphalitis risk cut-off points were >15.92, 0.412 cu or < 83.6 pmol/l.

The diagnostic value of an enlarged navel (a marker of perinatal fetoplacental circulation disorder in cows) for prediction of calf omphalitis was excellent (AUC = 0.953): sensitivity = 84.6%, specificity 100.0% at a cut-off point of 17.5 mm.

Respiratory diseases in calves born of preeclampsia-affected cows were due to endogenous intoxication, functional failure of the fetoplacental system, and circulatory disorders. Neonatal bronchopneumonia predictors are the intoxi-

cation coefficient, the MSP and estradiol content of the maternal blood serum, and the navel diameter within three hours post-birth. These indices had a very good, good, or excellent diagnostic value (AUC = 0.812; 0.782; 0.707 and 0.907), high sensitivity (85.7; 85.7; 77.8; and 88.9%), and specificity (59.1; 81.8; 77.3 and 77.3%). Bronchopneumonia risk cut-off points were 18.08 and 0.555 cu, < 71.2 pmol/l and > 17.5 mm. The greatest value for diagnosing early (Week 1) bronchitis in calves was attached to the estradiol (AUC = 0.729) and progesterone/estradiol ratio (AUC = 0.750) in maternal blood serum: the sensitivity was 70.8 and 54.2%; the specificity was 85.7 and 85.7%; cut-off points were < 116.8 pmol/l and > 571.0:1.

For predicting severe gastroenteritis in calves, the concentration of estradiol (AUC = 0.710) and the progesterone/estradiol ratio (AUC = 0.734) in cow serum, as well as the calf navel diameter within three hours post-birth (AUC = 0.782), were of greatest value: these indicators had a sensitivity of 77.8, 77.8, and 61.1%; a specificity of 77.3, 71.4, and 85.7%; and cut-off points of < 103.7 pmol/l, > 413.9:1, and > 17.5 mm.

Anemia in calves was mainly associated with the functional failure of the fetoplacental system. ROC analysis showed that the concentration of estradiol, DHEA-S, and the progesterone/estradiol ratio in preeclampsia-affected cows could predict severe neonatal anemia in calves at hemoglobin < 70 g/l. These indices had good diagnostic value (AUC = 0.782; 0.750, and 0.750), sensitivity (100.0% for all indices) and specificity (61.3; 71.0; and 51.6%); cut-off points for severe anemia in calves were < 78.0 pmol/l, < 0.229 μmol/l, and > 510.1:1.

Urine protein concentrations in preeclampsia-affected cows also had a good diagnostic value (AUC = 0.758) for prediction of neonatal anemia in calves. At low sensitivity (20.0%), this indicator had high specificity (94.7%); the cut-off point was > 2.0 g/l.

**3. Body weight of red-motley calves: normal pregnancy vs preeclampsia** (Novousmansky District, Voronezh Province, 2016)

| Indicator | *M*±SD | min-max | *Me* |
|---|---|---|---|
| Body weight, Day 1, kg | 39.4±6.4 | 27.0-50.0 | 40.0 |
| | 40.3±3.7 | 35.0-47.0 | 40.0 |
| Body weight, Day 30, kg | 47.2±7.9 | 28.0-59.0 | 48.0* |
| | 52.7±5.0 | 44.0-60.0 | 53.0 |
| Absolute body weight gain over Month 1, kg | 7.7±3.3 | 1.0-13.0 | 7.0** |
| | 12.3±2.2 | 7.0-15.0 | 13.0 |
| Relative body weight gain over Month 1, % | 19.6±8.8 | 3.7-38.2 | 17.5** |
| | 30.6±5.3 | 17.9-36.1 | 30.6 |
| Daily average body weight gain over Month 1, g | 254.7±106.6 | 33.0-433.0 | 233.0** |
| | 410.8±72.6 | 233.0-500.0 | 433.0 |
| Body weight, Day 60, kg | 62.6±8.4 | 38.0-75.0 | 64.5 |
| | 68.6±6.4 | 59.0-78.0 | 68.0 |
| Absolute body weight gain over Month 2, kg | 15.1±2.5 | 10.0-19.0 | 16.0 |
| | 17.2±1.9 | 13.0-20.0 | 18.0 |
| % Relative body weight gain over Month 2, % | 32.1±5.9 | 22.8-43.9 | 31.4 |
| | 33.6±3.1 | 28.2-36.7 | 35.1 |
| Daily average body weight gain over Month 2, g | 490.5±79.0 | 333.0-633.0 | 483.0 |
| | 573.2±63.3 | 433.0-660.0 | 660.0 |

N o t e. Values above the line are those of preeclampsia-affected cows ($n$ = 31); values below the line are those of the normal-pregnancy group ($n$ = 14)
* and ** Inter-group difference is statistically significant at $p < 0.05$ and $p < 0.01$, respectively.

Groups I and II did not differ significantly in body weight on Day 1. On Day 30, calves from preeclampsia-affected cows weighed 9.4% less ($p < 0.05$) than those born after a normal pregnancy; by Day 60, the difference in live mass leveled, see Table 3. The absolute body weight gain over the first month of life was 46.2% lower in Group I calves ($p < 0.01$), while the relative and daily average gain was 42.8% ($p < 0.01$) and 46.2% ($p < 0.01$) than the median values of

Group II offspring. Correlation analysis identified a statistically significant correlation of daily average body weight gain over the first month of life and endogenous intoxication indices: intoxication coefficient ($r_S = -0.79$ at $p < 0.01$), blood MSP ($r_S = -0.73$ at $p < 0.01$), estradiol concentration ($r_S = +0.37$ at $p < 0.05$) and progesterone/estradiol ratio ($r_S = -0.50$ at $p < 0.01$) in maternal serum, which reflects the functional status of the fetoplacental system. An inverse correlation of the daily average weight gain in calves and endogenous intoxication indices in preeclampsia-affected cows: intoxication coefficient ($r_S = -0.58$ at $p < 0.01$) and blood MSP concentration ($r_S = -0.57$ at $p < 0.01$) was observed over the second month, too.

Researchers' interest in such pregnancy pathologies as a functional failure of the fetoplacental system, intrauterine embryo maldevelopment and preeclampsia is attributable not only to the high incidence of these in productive animals [2, 4, 25] but also to their negative impact on the postnatal growth and health of the offspring [9, 13, 16]. According to the concept of developmental origins of health and disease, DOHaD, this or that factor present during critical intrauterine fetal development may have remote consequences in the postnatal ontogenesis [26-28]. Other researchers have earlier noted that preeclampsia-affected cows give birth to less viable calves [2, 29, 30] that have a higher incidence of omphalitis [12], gastrointestinal diseases [31], and respiratory diseases [18, 32]. However, it is not clear yet how maternal preeclampsia actually affects the morphofunctional status of newborn calves [2, 30].

The data presented herein suggest that preeclampsia-affected cows differ significantly in terms of urine protein concentration, endogenous intoxication indices (EAC/TAC, MSP, IC) and steroid hormone concentrations (progesterone, estradiol, DHEA-S) in serum, which affects the neonatal condition of their calves. ROC analysis proves that the intoxication coefficient and MSP concentration in the serum of preeclampsia-affected cows can be used as predictors of neonatal omphalitis and bronchopneumonia in calves. This data is consistent with earlier reports [33, 34] and indicates a close pathogenetic correlation of neonatal omphalitis and respiratory diseases in calves. It is known that MSPs can block cell receptors, load the active centers of the albumin molecule, and compete against regulatory peptides, which adversely affects humoral regulation and is toxic for the developing fetus [23, 25, 35]. The authors have shown that intrauterine intoxication negatively affects the daily average body weight gain in calves within the first two months of life: calves born of normal pregnancy had 46.2% gain over the first month ($p < 0.01$) than their counterparts born of preeclampsia-affected cows. The research has identified an inverse correlation of intoxication coefficient in preeclampsia-affected cows and the daily average body weight gain of their calves over the first month of life ($rS = 0.79$ at $p < 0.01$) or second month of life ($rS = 0.58$ at $p < 0.01$).

Finding the content and ratio of steroid hormones in the serum of deep-bone cows can identify the functional failure of the fetoplacental system [25, 36] to predict neonatal diseases in the offspring [4, 37]. Finding the estradiol concentration and progesterone/estradiol ratio in the serum has proven useful for predicting severe gastroenteritis and anemia in calves. Earlier, Lavrijsen et al. [38] experimented in vitro to demonstrate the dose-dependent progesterone effects on the fetal erythropoiesis in cattle, while Safonov et al. [37] showed how neonatal respiratory diseases in calves could be predicted on the basis of the progesterone and estradiol content and ratio in maternal serum.

Proteinuria in cows approaching labor indicates a functional disorder of the kidneys [2, 4, 10, 39]. This research has identified a high specificity of urine protein concentration in deep-bone cows (94.7%) for predicting anemia in calves

(cut-off at >2.0 g/l); this indicator is recommendable for use at farms.

It is known that preeclampsia in cows is associated with fetoplacental circulation disorders and the altered diameter and structure of umbilical vessels [4, 10-12]. Zolotaryov et al. [12] found that if a calf has a navel diameter of > 18.00 mm at the base within the first three hours post-birth, this retrospectively indicates fetoplacental circulation disorders and a high probability of omphalitis. In this study, this parameter did differ significantly in normal-birth vs preeclampsia groups ($p < 0.01$). The diagnostic value of an enlarged navel for prediction of calf omphalitis was excellent (AUC = 0.959): sensitivity = 84.6%, specificity 100.0% at a cut-off point of 17.5 mm. Note that the correlation of the navel diameter in newborn calves and the likelihood of gastroenteritis or bronchopneumonia, as well as the severity of these diseases as described in this report, was first identified by this research and is highly significant ($p < 0.01$). This research proves that calves are rendered susceptive to neonatal gastrointestinal and respiratory diseases not by omphalitis, but by deep morphofunctional changes in the mother-placenta-fetus system in preeclampsia, which precedes omphalitis.

Thus, determining the endogenous intoxication indices (medium-sized peptide content, effective albumin concentration, and the intoxication coefficient), the concentration of steroid hormones (progesterone, estradiol, and dehydroepiandrosterone sulfate) in the serum, and protein concentration in urine in preeclampsia-affected cows 25 to 32 days before expected calving helps not only objectively evaluate the animal's condition but also predict neonatal diseases and body weight gain in their offspring. Measuring the navel diameter at the abdominal wall base within three hours post-birth must be a mandatory part of the clinical examination protocol, as exceeding 17.5 mm in this value indicates a high probability of omphalitis (84.6% sensitivity and 100.0% specificity), severe gastroenteritis (61.1% and 85.7%), and bronchopneumonia (88.9% and 77.3%). The viability and health of calves born of dairy cows cannot be improved without timely prevention and treatment of preeclampsia, which must include metabolism corrections, reducing the endogenous intoxication and oxidative stress, and treating extragenital diseases.

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