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Original article

FETAL GROWTH RESTRICTION IN COWS IS ASSOCIATED WITH INTRAUTERINE DISELEMENTOSIS

V.A. Safonov, T.S. Ermilova, A.E. Chernitskiy

Abstract

Fetal growth restriction (FGR) is prevalent in highly productive dairy herds and presents a considerable challenge for animal husbandry. One contributing factor to FGR is the deficiency of essential trace elements and impaired placental transport functions in pregnant cows. In this study, we performed a comparative analysis of 12 trace elements and their ratios in the hair of newborn calves with a history of FGR (Group I, n = 18) and those born to cows with a normal pregnancy (Group II, n = 24). FGR was diagnosed based on ultrasound examinations of the pregnant cows performed at 38–45, 60–65, and 110–115 days of gestation using an Easi-Scan-3 scanner with a 4.5–8.5 MHz linear sensor (BCF Technology Ltd., Great Britain) following a previously established and published protocol. Hair samples from the calves were collected from the tail switch immediately before their first colostrum feeding. The concentrations of arsenic, cadmium, cobalt, copper, iron, mercury, manganese, molybdenum, nickel, lead, selenium, and zinc in the hair were analyzed using inductively coupled plasma mass spectrometry (Nexion 300D, Perkin Elmer, USA). To evaluate intrauterine diselementosis based on the trace element levels in the hair, various ratios were calculated: arsenic/selenium, mercury/selenium, lead/selenium, lead/zinc, cadmium/selenium, nickel/zinc, and iron/copper. Calves in Group I had significantly higher levels of cadmium in their hair (increased by 66.7%, $P < 0.05$) and mercury (increased by 15.0 times, $P < 0.05$) along with lower levels of copper (decreased by 30.7%, $P < 0.05$), selenium (decreased by 28.8%, $P < 0.05$), and zinc (decreased by 26.4%, $P < 0.05$) compared to calves in Group II. The concentrations of other trace elements in the hair did not differ significantly between the groups. These findings indicate that fetal development in calves during the last trimester of pregnancy occurs under conditions of an imbalance of essential and toxic trace elements. The mercury/selenium ratio in the hair of Group I calves was increased by 45.3 times (P < 0.05) compared to Group II calves, while the lead/selenium ratio was 2.81 times higher (P < 0.05), the cadmium/selenium ratio was 6.63 times higher (P < 0.05), the nickel/zinc ratio was 2.91 times higher (P < 0.05), and the iron/copper ratio was 2.64 times higher (P < 0.05). In this study, we also examined the potential causes and mechanisms underlying these imbalances.

Keywords: Fetal growth restriction; Cattle; Hair analysis; Trace elements

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Научная статья

ЗАДЕРЖКА РОСТА ПЛОДА У КОРОВ АССОЦИИРОВАНА С ВНУТРИУТРОБНЫМ ДИСЭЛЕМЕНТОЗОМ

В.А. Сафонов, Т.С. Ермилова, А.Е. Черницкий

Аннотация

Задержка роста плода (ЗРП) у коров широко распространена в высокопродуктивных молочных стадах и представляет серьезную проблему для животноводства. Одним из факторов, предрасполагающих к ЗРП, является дефицит эссенциальных микроэлементов и нарушения транспортной функции плаценты у беременных. В настоящей работе проведен сравнительный анализ содержания 12 микроэлементов и их соотношения в волосах у новорожденных телят с ЗРП в анамнезе (Группа I, n = 18) и особей с физиологическим течением беременности у их матерей (Группа II, n = 24). ЗРП диагностировали по результатам ультразвукового исследования матерей на 38-45, 60-65 и 110-115 дни гестации с помощью сканера «Easi-Scan-3» с линейным датчиком 4,5-8,5 МГц (BCF Technology Ltd., Великобритания) по ранее разработанному и опубликованному протоколу. Образцы волос у телят получали из кисти хвоста непосредственно перед 1-м кормлением молозивом. Методом масс-спектрометрии с индуктивно-связанной плазмой (Nexion 300D, Perkin Elmer, США) в образцах волос исследовали содержание мышьяка, кадмия, кобальта, меди, железа, ртути, марганца, молибдена, никеля, свинца, селена и цинка. Для оценки внутриутробного дисэлементоза по измеренному содержанию микроэлементов в волосах рассчитывали их соотношения: мышьяк/селен, ртуть/селен, свинец/селен, свинец/цинк, кадмий/селен, никель/цинк и железо/медь. У телят Группы I установлено повышенное содержание в волосах кадмия (на 66,7%, $P < 0,05$) и ртути (в 15,0 раз, $P < 0,05$) и пониженное – меди (на 30,7%, $P < 0,05$), селена (на 28,8%, $P < 0,05$) и цинка (на 26,4%, $P < 0,05$) по сравнению с животными Группы II. Содержание в крови других исследованных микроэлементов в волосах достоверно не различалось между группами. Результаты исследования показывают, что развитие плода у ЗРП-коров в последнем триместре беременности происходит в условиях дисбаланса эссенциальных и токсичных микроэлементов. Так, соотношение ртуть/селен в волосах у телят Группы I по сравнению с новорожденными Группы II было повышено в 45,3 раза ($P < 0,05$), свинец/селен – в 2,81 раза ($P < 0,05$), кадмий/селен – в 6,63 раза ($P < 0,05$), никель/цинк – в 2,91 раза ($P < 0,05$), железо/медь – в 2,64 раза ($P < 0,05$). Анализируются возможные причины и механизмы, лежащие в основе этих нарушений.

Ключевые слова: задержка роста плода; крупный рогатый скот; анализ волос; микроэлементы

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Introduction

Fetal growth restriction (FGR), formerly called intrauterine growth retardation [18; 58], is a disorder in which the embryo/fetus does not achieve its full development potential during gestation [1; 12; 18; 32]. This pathology is common among dairy cattle, affecting approximately one in four to even one in two pregnant cows [1; 42; 58]. The cause of its occurrence is placental insufficiency, which occurs when the mother is exposed to negative factors during the formation of the placenta or other factors that cause nutritional deficiency in the fetus [9; 42; 48; 55]. The main symptom of FGR during intrauterine diagnosis is the small size and disproportionate growth of fetal organs that occur under nutrient deficiency [1; 18; 43; 58]. In this case, the metabolism of the fetus is rearranged in such a way as to ensure the development of the brain to the detriment of other organs and tissues such as muscles, liver, and kidneys [11; 21; 33; 58].

Several factors influence the complex polycausal interaction between the fetus and the placenta, ultimately resulting in the development of FGR [42, 58]. These include endogenous (e.g., genetic abnormalities, endocrine and immune diseases) and exogenous (e.g., environmental stressors such as heat, bacterial and viral infections, intoxication, unbalanced or inadequate nutrition) factors [13; 25; 26; 42]. Recent research indicates that impaired mineral nutrition may be one of the most common causes of FGR in highly productive cows [13; 52; 53; 56], particularly in natural and man-made biogeochemical provinces [27; 49; 46; 51]. Selenium, copper, zinc, and cobalt are essential minerals for the normal development of the embryo and fetus in cattle [9; 24; 35; 45]. In the "mother–placenta–fetus" system, these minerals play crucial roles [8; 28; 30; 41]. Selenium has antioxidant and immunomodulatory functions, promotes detoxification of the body, influences the metabolism of zinc and iodine, and participates in the metabolism of vitamins [16; 20; 36; 51]. It promotes the oxidation of substances with the formation of oxides and peroxides, followed by the binding and excretion of active metabolites, protecting the body from excess cadmium, lead, and thallium [3; 7; 23; 36]. Selenium participates in various metabolic processes, is part of glutathione peroxidase, and influences the metabolism of thyroid hormones and sulfur-containing amino acids [36; 41; 44; 51]. Selenium deficiency in animals lowers immunity, results in the accumulation of degenerative changes in muscle tissue, and causes metabolic disorders, affecting the metabolism of proteins, lipids, and minerals [35; 36; 41; 47]. Selenium deficiency can develop into toxic liver dystrophy, cows will show reproductive function disorders, and newborn calves will experience slowed growth and increased respiratory diseases [28; 29; 44; 51]. Copper is part of many vitamins, hormones, and enzymes; it affects the elasticity of blood vessels, skin, cartilage, and bones by participating in the synthesis of elastin and collagen; it is part of the myelin sheath of nerves; and it binds microbial toxins [7; 9; 11]. Copper exhibits antioxidant properties and is important in synthesizing hemoglobin and sex hormones [7; 11]. Copper deficiency leads to the suppression of the expression of metallothionein 1A and a decrease in the activity of superoxide dismutase and cytochrome-C oxidase [7; 9]. Copper deficiency negatively affects the functional state of the brain, lungs, heart, and fetal skeleton [9; 11; 34]. Furthermore, copper deficiency can manifest in a decrease in reproductive function, including ovarian dysfunction and embryonic mortality, the appearance of microcytic hypochromic anemia, and the development of distress syndrome in the first months of life [10; 24; 28; 40]. Symptoms of insufficient copper intake in an animal's body can include weakness, bone fragility, lameness, joint enlargement and nodularity, thyroid gland enlargement, and increased respiratory diseases in neonatal calves [9; 11; 29; 49]. Zinc deficiency exacerbates these effects, affecting the work of ZIC genes necessary for the normal formation of the cerebellum, altering the distribution of natural killer cells-1 and connexin 43 in the myocardium, and reducing the activity of several antioxidant enzymes [7; 9;

11]. As a trace element associated with the metabolism of copper, molybdenum, cobalt, and iodine, zinc is part of enzymes, promotes cell division and differentiation, is part of proteins, and contributes to the formation of T-cell immunity [7; 9; 13]. Zinc deficiency manifests as a lack of appetite, diarrhea, weight loss, growth retardation, increased frequency of colds, hair loss, and dermatitis, and in adult animals, it leads to a disruption of sexual function [7; 13; 19; 40]. Cobalt is important for the body because it participates in enzymatic processes and affects hematopoiesis along with iron and copper; low levels of cobalt in the body lead to impaired cardiovascular function, anemia, delayed offspring development, distorted appetite, and deterioration in the quality of hair and skin [7; 14; 24; 33]. Cobalt deficiency negatively affects hematopoiesis, muscle protein and nucleic acid synthesis, and the state of energy metabolism [7], resulting in fetal development disorders [9; 49; 52]. On the other hand, there are reports of the negative impact on fetal development of increased concentrations of arsenic, iron, nickel, and lead [9; 27; 46; 54]. It is worth noting that when symptoms of deficiency of one or more trace elements appear, various combinations of characteristic signs are usually observed in one animal [3; 19; 49].

One method for retrospectively evaluating the trace element nutrition of the fetus in cows is through chemical elemental analysis of hair samples collected from calves shortly after birth [6; 50; 52]. This approach not only assesses the intake levels of individual trace elements through the placental barrier but also evaluates their ratios in the developing fetus [5; 6].

The *aim* of this study was to perform a comparative analysis of the concentrations of trace elements (arsenic, cadmium, cobalt, copper, iron, mercury, manganese, molybdenum, nickel, lead, selenium, and zinc) and their ratios in the hair of newborn calves with a history of FGR versus those with a normal pregnancy in their mothers.

Materials and methods

This study was performed in the Ikryaninsky District of the Astrakhan Oblast (Lower Volga Region, Russia). To identify FGR, clinical ultrasound examinations were performed on Simental breed cows at 38–45, 60–65, and 110–115 days after fertilization using an Easi-Scan-3 ultrasound scanner (BCF Technology Ltd., UK) equipped with a 4.5–8.5 MHz linear sensor following a previously established and published protocol [1]. Based on the evaluation, 18 cows with delayed embryo/fetal growth (indicative of FGR) and 24 cows with a normal pregnancy (healthy cows) were included in the study. Samples of unpigmented hair were collected from the tail switch of the newborn calves immediately before their first colostrum feeding using stainless steel scissors sterilized with ethanol [50].

All hair samples obtained after preparation [38] were sent for analysis to an accredited laboratory (Micronutrients LLC, Moscow, Russia), which is an associated company of the International Union of Pure and Applied Chemistry (IUPAC). The laboratory performed the quantitative determination of trace elements (arsenic, cadmium, cobalt, copper, iron, mercury, manganese, molybdenum, nickel, lead, selenium, and zinc) using inductively coupled plasma mass spectrometry with a Nexion 300D device (Perkin Elmer, USA). To evaluate intrauterine diselementosis, the ratios of trace elements in the hair were calculated: arsenic/selenium, mercury/selenium, lead/selenium, lead/zinc, cadmium/selenium, nickel/zinc, and iron/copper [5].

Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corporation, USA). The results were presented as median, interquartile range (IQR, Q1–Q3), and minimum (Min) and maximum (Max) values. The significance of differences between samples was assessed using the Mann–Whitney U test for independent samples.

Results

The results of the clinical ultrasound examination of the embryo/fetus in cows at 38–45, 60–65, and 110–115 days of gestation are shown in Table 1.

Table 1.

Morphometric parameters of embryos and fetuses in FGR cases compared to those in normal pregnancies in cows

Parameter	FGR cows (n = 18)		Healthy cows (n = 24)	
	Min–Max	Median	Min–Max	Median
38–45 gestation days				
Coccygeal–parietal size, mm	13–16	14.0	17–25	19.6
Body diameter, mm	6–9	7.6	10–13	10.8
60–65 days of gestation				
Coccygeal–parietal size, mm	26–47	39.3	52–78	68.4
Body diameter, mm	13–16	14.8	17–24	19.2
110–115 gestation days				
Uterine horn diameter, cm	10–16	13.9	18–25	19.4
Placental size, mm	11–18	14.3	19–34	26.2
Vibration intensity of the middle uterine artery, %	38–50	44.4	80–95	85.7

Table 1 shows that in cows with FGR, the morphometric characteristics of the embryo/fetus, including the uterine horn diameter, placental size, and vibration intensity of the middle uterine artery, were considerably reduced compared to values observed in healthy cows at the same gestational stage.

Table 2 shows the content of trace elements in hair samples from the tail switch of newborn calves.

Table 2.

Hair trace element content in newborn calves, µg/g

Trace element	FGR calves (n = 18)	Healthy calves (n = 24)	Physiological interval [50]
Arsenic	0.095 (0.052–0.175)	0.114 (0.089–0.222)	0.020–0.107
Cadmium	0.010* (0.010–0.020)	0.006 (0.001–0.010)	0.006–0.012
Cobalt	0.027 (0.010–0.066)	0.050 (0.030–0.074)	0.040–0.090
Copper	5.00* (3.00–7.35)	7.22 (6.00–8.20)	5.50–8.68
Iron	50.4 (35.3–97.5)	34.5 (25.4–57.9)	30.3–57.2
Mercury	0.030* (0.020–0.030)	0.002 (0.002–0.005)	0.020–0.030
Manganese	9.64 (8.56–12.2)	8.90 (7.03–12.4)	8.20–9.54
Molybdenum	0.037 (0.019–0.070)	0.021 (0.017–0.045)	0.017–0.067
Nickel	1.40 (0.455–3.29)	1.19 (0.390–1.97)	0.215–1.17
Lead	1.53 (1.20–2.78)	1.13 (0.525–1.38)	0.060–1.00
Selenium	0.289* (0.166–0.359)	0.406 (0.270–0.458)	0.180–0.400
Zinc	88.0* (34.0–121.7)	119.5 (94.0–126.9)	92.4–131.7

The data are presented as median and interquartile range (Q1–Q3).
* Differences with the group of healthy calves are significant at $P < 0.05$.

Compared to healthy newborns, FGR calves exhibited significantly higher levels of cadmium in their hair (increased by 66.7%, $P < 0.05$) and mercury (increased by 15.0 times, $P < 0.05$) along with lower levels of copper (decreased by 30.7%, $P < 0.05$), selenium (decreased by 28.8%, $P < 0.05$), and zinc (decreased by 26.4%, $P < 0.05$). The concentrations of other trace elements in the hair of newborns did not show significant differences between the groups.

Compared to the physiological range [50], 55.6% of FGR calves had reduced concentrations of cobalt in their hair, 61.1% had reduced copper, 22.2% had reduced selenium, and 55.6% had reduced zinc. Conversely, the levels of iron and manganese in the hair of FGR calves were elevated beyond the physiological range [50] in 38.9% and 50.0% of cases, respectively. In healthy newborn calves, reduced levels of cobalt, copper, selenium, and zinc in hair were observed 1.8–4.9 times less frequently. Both groups of calves exhibited excessive accumulation of arsenic, nickel, and lead in the hair during the final months of intrauterine development, with these accumulations being more pronounced in FGR cases. The mercury content in the hair of healthy calves was reduced, whereas in FGR calves, it was at the upper limit of the physiological range [50]. Thus, FGR and healthy calves exhibited significantly different trace element concentrations in their hair (Table 3). Specifically, the mercury/selenium ratio in the hair of FGR calves was 45.3 times higher ($P < 0.05$), the lead/selenium ratio was 2.81 times higher ($P < 0.05$), the cadmium/selenium ratio was 6.63 times higher ($P < 0.05$), the nickel/zinc ratio was 2.91 times higher ($P < 0.05$), and the iron/copper ratio was 2.64 times higher ($P < 0.05$), compared to those in calves with normal intrauterine development.

Table 3.

Ratios of trace element concentrations in the hair of newborn calves

Ratio	FGR calves (n = 18)	Healthy calves (n = 24)
Arsenic/selenium	0.235 (0.154–0.657)	0.306 (0.192–0.541)
Mercury/selenium	0.136* (0.098–0.182)	0.003 (0.002–0.006)
Lead/selenium	2.78* (2.14–5.00)	0.99 (0.83–1.46)
Lead/zinc	0.061 (0.029–0.112)	0.028 (0.011–0.059)
Cadmium/selenium	0.053* (0.046–0.080)	0.008 (0.001–0.017)
Nickel/zinc	0.032* (0.013–0.046)	0.011 (0.007–0.019)
Iron/copper	12.3* (7.74–18.0)	4.67 (3.10–11.5)

The data are presented as median and interquartile range (Q1–Q3).

* Differences with the group of healthy calves are significant at $P < 0.05$.

Discussion

The Lower Volga Region is a natural biogeochemical province characterized by low levels of selenium, copper, and cobalt in soils and pasture plants [2; 4; 57]. This poses a specific challenge for local cattle breeding because animals in this region receive these essential trace elements in lower concentrations compared to cattle in areas with more adequate soil levels [2; 17; 19]. Despite seasonal variations in trace element concentrations, the amounts available do not fully meet the animals' needs [2; 57], leading to a disruption in elemental homeostasis, a condition referred to in modern literature as "diselementosis" [29; 53]. Previous studies [2] have identified reduced concentrations of selenium and cobalt in lactating Simmental cows bred in the biogeochemical conditions of the Lower Volga Region despite adequate copper supply.

In this study, we evaluated the trace element supply to the fetus during the last trimester of pregnancy in Simmental cows from the Astrakhan region. One method for assessing fetal trace element nutrition is analyzing the elemental composition of tail switch hair collected from calves shortly after birth [6; 50; 52]. This analysis provides a retrospective view of not only the levels of specific trace elements transferred through the placental barrier but also their ratios in the developing fetus during the final trimester of pregnancy [6; 50]. Of the 42 calves examined, 18 (42.9%) had reduced levels of cobalt compared to the previously established physiological range [50], 14 (33.3%) had reduced levels of copper, 7 (16.6%) had reduced levels of selenium, and 15 (35.7%) had reduced levels of zinc. Concurrently, an increased content of arsenic, nickel, iron, and manganese was observed in the hair of most newborns (Table 2).

It is generally accepted that the intrauterine supply of trace elements crucial for the normal formation and development of the fetus in cattle is entirely dependent on the maternal intake and availability of these elements [6; 8; 34; 53]. Our data indicate that when pregnant cows have low levels of cobalt, copper, and selenium, their fetuses also suffer from deficiencies in these trace elements. Furthermore, inadequate maternal intake of these essential elements can result in the fetus accumulating excessive amounts of potentially toxic elements [3; 6; 9; 15]. Selenium is known as an antagonist of mercury and arsenic, and it helps protect the body from the excessive accumulation of lead and cadmium [7]. When zinc levels are low, the body tends to accumulate higher levels of cadmium, lead, and nickel [3; 7; 39]. Additionally, a deficiency in vitamin C, which is frequently observed in cows in the Lower Volga Region [57], can exacerbate the accumulation of nickel in the body [7].

It is well-established that inadequate micronutrient nutrition during critical fetal development periods adversely affects the formation of muscle and adipose tissue, endocrine regulation, and postnatal survival [9; 11; 21; 22; 41].

In this study, we assessed the morphometric characteristics of the embryo/fetus and placenta in experimental cows using an ultrasound scanner at 38–45, 60–65, and 110–115 days of pregnancy (Table 1). FGR was diagnosed in 18 out of 42 (42.9%) animals. Our findings indicate that the concentrations of essential and toxic elements in the hair of FGR calves significantly differ from those in calves without this condition (Table 2). Specifically, FGR calves showed a significant increase in cadmium and mercury levels in their hair, along with a tendency for excessive accumulation of iron, lead, and nickel, while also exhibiting deficiencies in copper, selenium, and zinc.

A previous study on red-mottled calves in the Chernozem Region (Voronezh Oblast, Russia), where trace element levels in soils and pasture plants are considered optimal [52], found that FGR calves had lower concentrations of cobalt, copper, manganese, selenium, and zinc in their hair compared to newborn calves with normal physiological development. In our study, statistically significant differences were observed between calf groups in the levels of copper, selenium, zinc, cadmium, and mercury but not in cobalt and manganese (Table 2). This could be attributed to the specific biogeochemical conditions of the Lower Volga Region and the unique trace element exchange dynamics in the “mother–placenta–fetus” system of Simmental cattle. Comparison with the physiological reference values [50] revealed that 55.6% of FGR calves had low cobalt levels in their hair, 61.1% had low copper, 22.2% had low selenium, and 55.6% had low zinc. In cases of normal fetal development, deficiencies of these trace elements were observed 1.8–4.9 times less frequently.

The results indicate that fetal development in FGR cows during the last trimester of pregnancy occurs amidst an imbalance of essential and toxic trace elements. Intrauterine diselementosis in FGR calves is confirmed by significant changes in the ratios of trace elements in their hair (Table 3). Specifically, the mercury/selenium ratio in the hair of FGR newborn calves was 45.3 times higher ($P < 0.05$), the lead/selenium ratio was 2.81 times higher ($P < 0.05$), the cadmium/selenium ratio was 6.63 times higher ($P < 0.05$), the nickel/zinc ratio was 2.91 times higher ($P < 0.05$), and the iron/copper ratio was 2.64 times higher ($P < 0.05$) compared to those in calves with normal intrauterine development.

Our data reveal considerable disruptions in trace element metabolism in FGR calves during the final months of intrauterine development. It is likely that inadequate levels of essential trace elements, coupled with endocrine and metabolic disorders associated with FGR [31; 37; 43], create conditions where the fetus cannot fully neutralize the increased intake of heavy metals from the mother’s blood. This observed imbalance between essential and toxic trace elements negatively impacts the formation of muscle and adipose tissue, metabolism, endocrine regulation, and hematopoiesis in the fetal body [9; 11; 22; 41].

In FGR calves, common postnatal symptoms include anemia, hypoimmunoglobulinemia, dyslipidemia, hypoglycemia, lactic acidosis, and deficiencies in the antioxidant system, all stemming from intrauterine diselementosis [29; 37; 52; 58]. Our findings highlight severe disruptions in trace element metabolism in FGR calves during the final months of intrauterine development. Future studies should investigate the underlying causes and potential mechanisms of these disruptions.

Conclusion

The trace element profile of hair in FGR calves from the Lower Volga Region distinctly differs from that of newborn calves with normal physiological development. The findings suggest that an intrauterine imbalance of essential and toxic trace elements—such as deficiencies in copper, selenium, and zinc, along with excess levels of mercury, lead, cadmium, and nickel—may play a role in the development of FGR in cattle.

Ethics approval. The study adhered to all relevant international and national guidelines for animal care and use. The experimental design, including housing, feeding conditions, and all animal procedures (clinical assessments, ultrasound, and hair sampling for analysis), was approved by the Bioethics Commission of the All-Russian Scientific Research Veterinary Institute of Pathology, Pharmacology, and Therapy in Voronezh, Russia (Protocol No. 2-03/23 dated March 1, 2023).

Conflict of interest. The authors declare that they have no conflict of interest.

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