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Endocrine and metabolic mechanisms of embryo and fetal intrauterine growth retardation in dairy cows

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Abstract: This research was conducted to study hormonal and metabolic statuses and to determine ultrasound criteria for early diagnosis of fetal growth retardation syndrome (FGRS) in dairy cows of Black-motley (n = 41) and Red-motley (n = 56) Holstein breeds. Concentrations of progesterone, testosterone, 17β-estradiol, dehydroepiandrosterone sulfate, cortisol, and triiodothyronine in blood serum were assessed by immune-enzyme analysis. Concentrations of plasma L-ascorbic acid and nitrogen oxides, serum immune globulin, middle molecular peptides, and bactericidal and lysozyme activity were determined by spectrophotometry. On days 38–40 and 60–65 of gestation, FGRS in cows was associated with hypoprogesteronemia, decrease of adrenal and thyroid gland functions, decreased nitric oxide synthesis, low immune responsiveness, and high endogenous intoxication. In conclusion, body length less than 16 mm on days 38–40 and less than 45 mm on days 60–65 are the criteria for designating underdevelopment of embryos and fetuses in dairy cows. FGRS genesis in dairy cows is determined by the embryo's and fetus's malnutrition at the early implantation and placentation stages, caused by the imbalance of endocrine regulation, nitric oxide systems, and endogenous intoxication.

Key words: Fetal growth retardation syndrome, cow, ultrasound diagnostics, hormones, nitrogen oxides, L-ascorbic acid

1. Introduction

Potential increase in efficiency in modern dairy husbandry can result from decreases in antenatal and neonatal losses. One detriment to increased efficiency is embryo and fetal intrauterine growth retardation (FGRS), an inadequacy of sizes at specific periods of gestation. This pathology is rather widespread in both scientific literature and clinical practice and is an important cause for perinatal morbidity and fetal and litter mortalities (1–3). Moreover, manifestation of this syndrome has a negative impact on subsequent reproductive function of the dam (2) and postnatal formation and function of neonates' organs and digestive, respiratory, and reproductive systems, predisposing these animals to metabolic and endocrine diseases and decreased fertility (1,3–5).

The mechanisms leading to disorders in formation and growth processes in the embryo and fetus are determined mainly by nutrition provided during the first stages from endometrial gland secretory activity (6,7) and by the intensity of vascularization in the placenta and utero-placental blood circulation in the transition to placental circulation (1,8–10). However, inadequate knowledge regarding the role of the metabolic homeostasis of the mother to provide adequate formation of the mother–embryo–placenta–fetus biological system limits development of methods for managing these processes. Thus, the current research aimed to achieve early diagnosis of FGRS and to determine the role of hormonal and metabolic statuses of cows in the genesis of this pregnancy failure.

2. Materials and methods

2.1. Animal materials and study design

The research was performed during winter when cattle were stalled indoors. The study included cows of Black-motley (n = 41) and Red-motley (n = 56) Holstein breeds with an average annual milk production of 6500–7500 kg. Rations contained matched mono-fodders prepared according to the normative standards for animals' needs in essential nutrients and vitamin-mineral supplements. A

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total of 97 cows were used: 56 at days 38–40 and 41 at days 60–65 of gestation. The methods of transrectal palpation and echography with the use of ultrasound scanner Easi-Scan-3 with a linear transducer of 4.5–8.5 MHz (BCF Technology Ltd., UK) were applied for the measurement of genitals and embryos' and fetuses' metrical indices. Size and consistency of the parous uterine horn and the embryo's coccyx–parietal distance and diameter of the embryo were measured. Generalized literature and our own research data were used for estimating fetometric indices (11–13).

2.2. Collection of samples

After clinico-echographic examination, blood samples were taken via puncture of the jugular vein in 13 cows (5 Black-motley and 8 Red-motley animals) with normal physiological course of gestation and 16 cows (6 Black-motley and 10 Red-motley animals) with FGRS. These cows were sampled again at days 230–240 of gestation. Blood was collected into sterile vacuum tubes without anticoagulant or with EDTA to obtain blood plasma by centrifugation of the stabilized blood EDTA at 4000 × *g* for 10 min (UC-1612, ULAB, China) at room temperature. After clotting for 1 h at room temperature, blood samples without anticoagulant were centrifuged (UC-1612, ULAB) at 4000 × *g* for 10 min at room temperature, and sera were harvested carefully and stored at –20 °C until biochemical analyses.

2.3. Biochemical analyses

Quantitative analysis of progesterone, testosterone, 17β-estradiol, dehydroepiandrosterone sulfate (DHEAS), cortisol, and triiodothyronine in serum was conducted by immune-enzyme analysis test systems validated for cattle (Xema-Medica Co. Ltd., Russia) and an immunoenzyme reaction analyzer (Uniplan, AIFR-1, Pikon, Russia). Quantitative analysis of L-ascorbic acid and nitrogen oxides (NOx = NO^{2-} + NO^{3-}) in plasma and middle molecular peptides and immune globulin in serum was conducted by spectrophotometry (UV-1700, Shimadzu, Japan) according to the procedure modified from the relevant literature (14–17).

2.4. Analysis of serum bactericidal and lysozyme activity

Bactericidal and lysozyme activity of serum was evaluated according to the modified method of Voronin et al. (18). For the study of serum bactericidal activity, 1 mL of serum and 0.1 mL of 24-h *Escherichia coli* broth culture were added into 4.5 mL of Hottinger broth (test samples). Only microorganism culture was added to control samples. Tube contents were carefully agitated, 2 mL of the mixture was selected, and its optical density was determined at 490 nm (UV-1700, Shimadzu). The mixture left in the tubes was incubated at 37 °C (TS-1-80 SPU, Smolenskoye SKTB, Russia) for 3 h, and its optical density was measured repeatedly. Bactericidal activity of blood serum was expressed in optical density growth inhibition units in test samples in comparison with control samples (%). For quantitative analysis of serum lysozyme activity, tabulated values of dependence between lysozyme concentration and its lytic action on *Micrococcus lysodeikticus* culture, slurried in 0.06 M phosphate buffer (pH 7.2–7.4), were used (19).

2.5. Statistical analysis

For statistical analysis, the independent sample t-test was used to determine the difference between the values obtained from control (physiological gestation course) and FGRS groups. Statistical evaluation of differences between cows with gestation periods of 38–40, 60–65, and 230–240 days was done using the paired t-test in STATISTICA 8.0 (StatSoft. Inc., USA). Statistical significance was set at $P < 0.05$. All data were expressed as mean ± standard error of the mean (SEM).

3. Results

3.1. Metric values of embryo and fetus

Fetal growth retardation occurred in 36.7% of the Black-motley cows (n = 41) and in 42.9% of the Red-motley cows (n = 56). The maximum manifestation of this pathology was observed in first-lactation cows (45.0% in Black-motley cows and 54.5% in Red-motley cows), and the minimum manifestation was observed in cows in their third or above lactation (27.3% in Black-motley cows and 28.6% in Red-motley cows).

From Table 1, embryos with growth retardation at 38–40 days had linear sizes of 67.3% (13.8 ± 0.37 mm, $P < 0.001$) and corpus diameters of 72.3% (8.1 ± 0.42 mm, $P < 0.001$) of those of the controls. At the transition into fetal stage (60–65 days), the norm was characterized by linear sizes increasing 3.32 times to 68.1 ± 4.48 mm ($P < 0.001$) and corpus diameter increasing 1.68 times to 18.8 ± 2.35 mm ($P < 0.001$). The length of embryos with retarded growth processes was 77.8% of the length of the normally developing embryos ($P < 0.001$).

Fetal growth retardation among examined Black-motley breed cows (n = 41) was registered in 36.7% of them and among Red-motley breed cows (n = 56) in 42.9% of animals. The maximum manifestation of this pathology was marked in cows of the first lactation (45.0% in Black-motley breed cows and 54.5% in Red-motley breed cows) and the minimum manifestation of this pathology was marked in cows of the third or above lactations (27.3% in Black-motley breed cows and 28.6% in Red-motley breed cows).

3.2. Hormonal status

Concentration of progesterone in serum of cows with FGRS in comparison with the animals of the control group (physiological gestation course) appeared to be lower by 35.3% ($P < 0.01$) (Table 2).

Table 1. Metric values of embryos and fetuses of the cows with physiological gestation course and FGRS.

Parameters	Gestation period of 38–40 days		Gestation period of 60–65 days	
	Control group Mean ± SEM (min–max) (n = 31)	FGRS group Mean ± SEM (min–max) (n = 25)	Control group Mean ± SEM (min–max) (n = 24)	FGRS group Mean ± SEM (min–max) (n = 17)
Coccyx–parietal size, mm	20.5 ± 0.66 (18–25)	13.8 ± 0.37^{c} (12–16)	68.1 ± 4.48 (50–80)	38.3 ± 2.48^{c} (25–45)
Corpus diameter, mm	11.2 ± 0.59 (10–13)	8.1 ± 0.42^{c} (7–9)	18.8 ± 2.35 (17–24)	14.6 ± 0,67^{c} (12–16)

Statistical importance between control and FGRS groups: $^{c}P < 0.001$.

Table 2. The levels of hormones in blood serum in physiological gestation course and FGRS.

Parameters	Gestation period of 38–40 days Mean ± SEM	Gestation period of 60–65 days Mean ± SEM	Gestation period of 230–240 days Mean ± SEM
Progesterone (nmol/L)	13.9 ± 1.15 9.0 ± 0.79^{b}	24.9 ± 1.58*** 18.7 ± 1.80^{a***}	10.8 ± 0.76 16.4 ± 1.00^{c}
Testosterone (nmol/L)	1.46 ± 0.104 1.68 ± 0.190	1.57 ± 0.123 1.41 ± 0.052	2.43 ± 0.254 1.92 ± 0.062^{c}
17β-estradiol (nmol/L)	0.32 ± 0.031 0.43 ± 0.024	0.49 ± 0.042^{c***} 0.23 ± 0.013***	0.96 ± 0.073 0.54 ± 0.020^{c}
DHEAS (μg/ml)	0.11 ± 0.010 0.18 ± 0.013	0.19 ± 0.011*** 0.13 ± 0.012^{c***}	0.26 ± 0.013 0.23 ± 0.010^{c}
Cortisol (nmol/L)	6.34 ± 1.762 5.30 ± 0.410	15.70 ± 1.224*** 3.44 ± 0.320^{c*}	12.00 ± 0.824 6.50 ± 0.581^{c}
Triiodothyronine (nmol/L)	3.98 ± 0.323 3.72 ± 0.284	7.98 ± 0.642*** 2.56 ± 0.221^{c**}	5.12 ± 0.260 2.92 ± 0.150^{c}

Above the line: control group (n = 13), under the line: FGRS group (n = 16).
Statistical importance between control and FGRS groups: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.
Statistical importance between gestation periods of 38–40 and 60–65 days: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

In cows with normal course of physiological gestation, the embryo's transition into fetal development (60–65 days) in comparison to 38–40 days was accompanied by increased endocrine gland function: 79.1% in progesterone ($P < 0.001$), 53.1% in 17β-estradiol ($P < 0.001$), 147.6% in cortisol ($P < 0.001$), 72.7% in DHEAS ($P < 0.001$), and 100.5% in triiodothyronine ($P < 0.001$). Although progesterone concentration in cows with FGRS increased by 107.8% ($P < 0.001$), the other hormones, which are responsible for protein synthesis, bone formation, and proliferative changes in cows' uterine tissues, decreased: 17β-estradiol to 46.5% ($P < 0.001$), cortisol to 35.1% ($P < 0.05$), DHEAS to 27.8% ($P < 0.001$), and triiodothyronine to 31.2% ($P < 0.01$), respectively, in comparison with 38–40 days of gestation. Thus, the concentration was lower for progesterone by 24.9% ($P < 0.05$), 17β-estradiol by 53.1% ($P < 0.001$), cortisol by 78.1% ($P < 0.001$), DHEAS by 31.6% ($P < 0.001$), and triiodothyronine by 67.9% ($P < 0.001$) in blood serum of cows with FGRS on days 60–65 of gestation in comparison with the animals of the control group.

The concentrations of cortisol, DHEAS, 17β-estradiol, and testosterone in serum of cows with FGRS during the final gestation period (230–240 days) appeared to be 45.8% ($P < 0.001$), 11.5% ($P < 0.001$), 43.8% ($P < 0.001$), and 21.0% ($P < 0.001$) lower, respectively, than in the control group level. Progesterone concentration in serum of cows with FGRS, on the contrary, was 51.9% ($P < 0.001$) higher than in the controls. The progesterone/17β-estradiol ratio was 30.4 in FGRS cows to 11.2 in cows with physiological course of gestation. In addition, cows with FGRS had decreased ($P < 0.001$) triiodothyronine concentration, which was 57.0% of that of controls.

3.3. Immunological and biochemical indices

Cows with FGRS had 75.5% of the L-ascorbic acid in plasma on days 38–40 ($P < 0.05$), 76.7% on days 60–65 ($P < 0.01$), and 75.9% on days 230–240 ($P < 0.01$) of that in the control group. L-ascorbic acid decrease in the cows with FGRS also had a negative impact on their immunological status indices (Table 3); serum lysozyme activity in cows with FGRS was 60.7% ($P < 0.01$) at the stage of active embryo, 80.9% at the fetogenesis stage ($P < 0.05$), and 87.2% at the end of gestation ($P < 0.05$) compared to the control group. Serum immune globulin level in cows with FGRS throughout the gestation period was 19.0%–10.2% lower ($P < 0.05$) in comparison with the control group.

Concentration of nitrogen oxides in serum was lower (76.1%, $P < 0.05$) in cows with FGRS at the placentation stage (days 60–65) in comparison to the control group. Nitrogen oxide concentrations in blood serum of cows with FGRS at the final gestation stage, on the contrary, increased by 19.9% ($P < 0.05$) in comparison with the control group.

Significant increase of middle molecular peptides level in serum of cows with FGRS [by 28.6–50.0% ($P < 0.001$) in comparison with the indices of the control group] marked the presence of endogenous intoxication and activation of proteolysis processes.

4. Discussion

Embryo and fetal intrauterine growth retardation in cows is a polyfactorial syndrome (1,20), which is defined as an inadequacy of forming embryos and fetuses and their gestation periods. Clinical method of genital organ state evaluation (13) is used for FGRS diagnosis in pregnant cows. The results of the current research showed that the accuracy of FGRS diagnosis in pregnant cows significantly increased by determining the embryo's coccyx–parietal size by ultrasonography. On the basis of the literature (11,12) and the results of the present study, body length less than 16 mm on days 38–40 and less than 45 mm on days 60–65 are the criteria for designating underdevelopment of embryos and fetuses in cows.

It is conventional to think that the processes of the embryo's growth and development in cows are regulated mainly by the character of the maternal nutrition, metabolic homeostasis, and maternal genital organs state (1,21,22). The current work accentuated genetic factors.

The results show that the leading role in FGRS forming belongs to the nutritional factors of the forming embryo, its immunotrophic interaction with the mother's organism, and endogenous intoxication state.

At the early stages of embryo formation, growth retardation is connected with hypoprogesteronemia, determined by hypoplasia of the yellow body. Clinico-echographic data proved it. Thus, yellow body size in FGRS cows appeared to be 75.8% (13.8 ± 0.59 mm, $P < 0.001$) on days 38–40 and 79.0% (16.2 ± 0.59 mm, $P < 0.001$) on days 60–65 of gestation in comparison with controls. On one hand, progesterone deficit does not provide optimal secretory reaction of the uterine glands and the conditions for balanced nutrition and the embryo's implantation. On the other hand, it promotes aggressive increase of peripheral monocytes and lymphocytes in relation to both the embryo's tissues and the further forming placenta (7).

Table 3. Plasma L-ascorbic acid and nitrogen oxides levels, serum bactericidal activity, lysozyme activity, serum immune globulin, and middle molecular peptides of cows with FGRS and control groups.

Parameters	Gestation period of 38–40 days Mean ± SEM	Gestation period of 60–65 days Mean ± SEM	Gestation period of 230–240 days Mean ± SEM
L-ascorbic acid (μmol/L)	23.3 ± 1.36 17.6 ± 1.16[a]	23.2 ± 0.87 17.8 ± 0.91[b]	16.2 ± 0.93 12.3 ± 0.66[b]
Nitrogen oxides (μmol/L)	95.2 ± 3.90 101.2 ± 5.40	132.4 ± 10.80 100.7 ± 8.70[a]	75.7 ± 5.14 90.8 ± 6.25[a]
Serum bactericidal activity (%)	83.3 ± 2.42 77.9 ± 3.15	80.2 ± 3.22[c] 75.5 ± 2.60	75.4 ± 3.92 69.1 ± 2.75[c]
Serum lysozyme activity (μg/mL)	0.56 ± 0.021 0.34 ± 0.010[b]	0.68 ± 0.042 0.55 ± 0.031[a]	0.78 ± 0.022 0.68 ± 0.030[a]
Immune globulin (g/L)	29.4 ± 1.84 23.8 ± 0.89[a]	29.2 ± 1.48 27.4 ± 1.72[a]	32.2 ± 1.90 28.9 ± 2.11[a]
Middle molecular peptides (OD 210)	0.40 ± 0.02 0.56 ± 0.03[c]	0.56 ± 0.02 0.72 ± 0.03[c]	0.40 ± 0.01 0.60 ± 0.02[c]

Above the line: control group (n = 13), under the line: FGRS group (n = 16).
Statistical importance between control and FGRS groups: [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$.

Besides functional insufficiency of sexual glands in cows with FGRS, adrenal and thyroid gland function decreased, leading to hormonal imbalance.

At the stage of placentation the role of the embryo's nutrition belongs to placenta vascularization processes and uteroplacental blood circulation formation (1,8,10), the key regulator of which is nitric oxide (10,23–25). The current research demonstrated that the cows with FGRS at the stage of placentation had an evident $NO^{\cdot}$ deficit, which was proved by the decrease by 23.9% of its stable metabolites concentration (NO^{2-} + NO^{3-}) in blood serum ($P < 0.05$) in comparison with the control group (physiological gestation course). Decreased $NO^{\cdot}$ synthesis decelerates placental–embryonic blood flow, disturbs nutrient and oxygen transfer to the fetus, and restrains its growth (24).

A significant decrease of L-ascorbic acid level in blood plasma was observed throughout the whole research by 23.3%–24.5% in comparison with the animals from the control group. The decrease of the organism's provision with vitamin C had a negative impact on the immune status indices of the cows with FGRS, and in the first place on serum lysozyme activity, reflecting the functional state of the neutrophils. Serum lysozyme activity in cows with FGRS was significantly lower than the mean values in the control group throughout the whole gestation. Vitamin C also provides antioxidative protection for the developing embryo and fetus (26), and participates in the processes of the forming embryo's connective tissue (27,28). Thus, the decrease of its level in pregnant animals should be considered as an extremely unfavorable factor for the embryo's and fetus's development.

The results showed that a significant role in FGRS pathogenesis in cows belongs to endogenous intoxication, with a significant (1.3–1.5 times) increase of middle molecular peptides concentrations in blood serum in comparison with the control group ($P < 0.001$). Middle molecular peptides are formed as a result of proteolysis of serum and tissue proteins, and their levels in blood serum depend on both the state of detoxication systems and the activity of proteolysis processes. Being molecular analogs of regulatory peptides, short-chain peptides are capable of blocking cell membrane receptors, decreasing transporting abilities of albumin, and disturbing many metabolic processes (29). Moreover, endogenous intoxication can exhaust adaptive resources of cellular and humoral immunity (30) that we have registered in cows with FGRS.

Thus, the realization of the genetic programing and the fetus's development in dairy cows to a large extent is determined by the level of synthesis and metabolism of the sex hormones, corticosteroids, and thyroid hormones, which are specialized regulators of the biochemical and biophysical processes in the mother's and fetus's organisms. The key moment in the fetus's growth retardation syndrome formation is fetal malnutrition at the stage of implantation and early placentation, connected with incompleteness of endometrium secretory transformation and placental–embryonic blood flow, caused by the imbalance in sex steroid synthesis and the nitric oxide system. Decreased immunobiological reactivity level of the mother's organism and endogenous intoxication state appear as the determining factors causing functional insufficiency development in the forming biological mother–fetus system. The revealed pathogenetic mechanisms of formation, growth, and development disorders of the embryo and fetus may be the basis for developing effective prophylaxis and therapy strategies in cows with FGRS.

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