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METABOLIC STATUS OF THE COWS UNDER INTRAUTERINE GROWTH RETARDATION OF EMBRYO AND FETUS

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Abstract

Intrauterine growth retardation of embryo and fetus (IUGR) in cows is a polyfactorial syndrome which is defined as an inconsistency between the sizes of forming embryos and fetuses and gestation periods. It is thought that the processes of embryo and fetus growth and development in cows are determined by morphofunctional integrity of gametes entering into the process of insemination and are mainly regulated by the character of maternal nutrition, state of metabolic homeostasis and maternal genitals. This work was devoted to the study of cows' metabolic status under intrauterine growth retardation of embryo and fetus. In 2013 at a large dairy complex (Agrotekh-Garant Ltd. Nashchekino, Anninskii Region, Voronezh Province) a total of 53 Red-motley Holstein cows with average annual productivity of 6.0-6.5 thousand kg were studied for protein metabolism indices (content of total proteins, protein fractions, serum urea), carbohydrates (blood concentration of glucose, lactic and pyruvic acids), vitamins (A, E, C), hormonal homeostasis (blood serum content of progesterone, dehydroepiandrosterone sulfate, testosterone, estradiol, cortisol, triiodothyronine), endogenous intoxication (concentration of middle molecular peptides, urea, creatinine, transaminase blood serum activity) and nitric oxide system on the days 38-40, 60-65, 110-115 and 230-240 of gestation. The impact of these indices on the development of embryo and fetus was also studied. Blood was collected from the jugular vein in the morning before feeding. The evaluation of genitals and metrics of embryo and fetus were done by the method of transrectal palpation and sonography with the use of bovine ultrasound scanner Easi-Scan-3 with 4.5-8.5 MHz linear transducer (BCF Technology Ltd, United Kingdom). The diameter of the fertilized horn, placenta size, body diameter and coccyx-parietal size of the fetus were determined. Coccyx-parietal size of 12-16 mm and body diameter of 7-9 mm were the criteria of development at the age of 38-40 days, 25-45 mm and 12-16 mm — at the age of 60-65 days, respectively. The diameter of the fertilized horn of 9-15 cm and placenta of 10-17 mm were the criteria of development at the age of 110-115 days. The animals with IUGR, diagnosed during 38-40 days of gestation, were included into the experiment on the days 230-240. It is stated that during early stages of fetus formation (38-40 days) the retardation of its growth and development is connected with hypoprogesteronemia determined by hypoplasia of the yellow body. The cows with IUGR also demonstrated blood serum decrease of cortisol by 36.9 % ($p < 0.01$) and increase of triiodothyronine by 35.4 % ($p < 0.005$) in comparison with the animals with physiological gestation course that proves total hormonal imbalance. At the stage of placentation (60-65 days) the cows with IUGR demonstrated evident deficit of nitric oxide that was proved by the decrease of its stable metabolite concentration ($\text{NO}^{2-} + \text{NO}^{3-}$) in blood serum by 23.9 % ($p < 0.05$) in comparison with the level of the cows with physiological gestation. Authentic decrease of vitamin C content in blood serum, increase of middle molecular peptides level and activity of γ -glutamyl transferase by 42.9-51.0 %, 32.6-67.7 % and 22.1-54.0 % ($p < 0.01$), respectively, in comparison with the animals with physiological gestation were observed in cows with IUGR throughout the research. The article discusses the role of metabolic disorders in pathogenesis of intrauterine growth retardation of embryo and fetus in cows.

Keywords: cows, gestation, intrauterine growth retardation of embryo and fetus, metabolism, hormones, nitric oxide, vitamin C, middle molecular peptides, γ -glutamyl transferase

Intrauterine growth retardation of fetus (IUGR) is quite widespread in farm animals and has a negative impact on the viability of the fetus, the resulting offspring, the act of birth and postpartum period in parturient and postpartum

cows [1-3]. In addition, IUGR syndrome adversely affects postnatal ontogenesis [4, 5] and morphological and functional formation of digestive [5], respiratory [6], and reproductive organs [7]. Predisposition to metabolic and endocrine diseases [5, 8], reduced fertility and productivity [7, 9] are observed in the animals and their offspring. Therefore, IUGR remains one of the main problems of productive animal reproduction, being a considerable reserve to increase the efficiency of modern animal husbandry.

It is known that the normal course of pregnancy, growth, and development of embryo and fetus are determined by morphological and functional usefulness of gametes involved in fertilization, and by the state of hormonal and metabolic homeostasis of the mother's body [5, 8, 10-12].

For the first time, we performed a comparative analysis of the metabolic profile of Red-motley cows at physiological gestation and intrauterine growth retardation of embryo and fetus at days 38-40, 60-65, 110-115, and 230-240 of gestation. We studied the main parameters of protein (total protein, protein fractions, serum urea), carbohydrate (blood concentrations of glucose, lactic and pyruvic acids), and vitamin (A, E, C) metabolism, hormonal homeostasis (serum progesterone, DHEAS, testosterone, estradiol, cortisol, triiodothyronine), endogenous intoxication (serum concentrations of middle molecular weight peptides, urea, creatinine, and transaminases), and nitric oxide system.

The purpose of this research was to study the metabolic status of cows with the syndrome of intrauterine growth retardation of fetus during gestation.

Technique. Research was performed in the winter-stall period of 2013 at Agrotekh-Garant LLC Nashchekino (Annino Region, Voronezh Province) on Red-motley leashed cows with an average annual productivity of 6.0-6.5 thousand kg. A total of 53 cows were examined, including 11 on days 38-40, 18 on days 60-65, 11 on days 110-115, and 13 (of those with IUGR diagnosed at days 38-40) on days 230-240 of gestation.

Examination of genitals and metrics of embryo and fetus was performed by transrectal palpation and sonography using Easi-Scan-3 ultrasound scanner (BCF Technology Ltd., United Kingdom) with 4.5-8.5 MHz linear transducer. Fertilized horn diameter, placenta size, fetal body diameter and parietal-coccygeal size were measured. The criteria of growth retardation were the coccyx-parietal size of 12-16 mm and body diameter of 7-9 mm at the age of 38-40 days, and 25-45 mm and 12-16 mm at 60-65 days, respectively; the diameter of fertilized horn of 9-15 cm and placenta of 10-17 mm were the criteria of growth retardation in 110-115 day old fetus [2].

Blood was sampled from the jugular vein in the morning. Serum concentrations of sex (progesterone, dehydroepiandrosterone sulfate — DHEAS, testosterone, estradiol-17 β), corticosteroid (cortisol), and thyroid (triiodothyronine) hormones were measured by ELISA using Hema-Medica (Hema-Medika LLC, Russia) test systems and an Uniplan AIFR-1 immunoassay reaction analyzer (Pikon JSC, Russia).

Serum and whole blood proteins, total immunoglobulins, urea, creatinine, vitamins A, E, C, glucose, lactic and pyruvic acids, inorganic phosphorus, total nitric oxide stable metabolites ($\text{NO}_x = \text{NO}_2^- + \text{NO}_3^-$), middle molecular weight peptides (MWP), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ -glutamyl transferase (GGT) activity were measured using a Hitachi-902 biochemical analyzer (Roche Diagnostics, Japan) and UV-1700 spectrophotometer (Shimadzu, Japan) by standardized assay of metabolic indicators [13-15].

Correlation analysis and *t*-test for independent variables (Statistica 8.0, Stat Soft Inc., USA) were used for data statistical processing.

Results. Intrauterine embryo growth retardation was observed in cows against the sexual and adrenal hormone synthesizing dysfunction and the hormonal homeostasis system imbalance (Table). First of all, this applies to the secretion of progesterone, the main pregnancy hormone which provides endometrium transformation and the trophic function of endometrial glands in relation to the growing fetus. Its blood concentration in these animals was 2.40 times lower (8.0 ± 0.9 nmol/l versus 19.2 ± 1.7 nmol/l, $p < 0.001$) as compared with normal gestation.

Corpus luteum is the main producer of progesterone in early pregnancy. Its insufficient activity manifested in hypoprogesteronemia does not provide an optimal secretory response of uterine glands and enough embryo nutrition, but contributes to the aggressive reaction of peripheral mononuclear cells (monocytes, lymphocytes) against the tissues of the growing placenta and the embryo itself [16-18].

The functional failure of ovarian corpus luteum in cows was followed by the decreased function of adrenal glands which produce glucocorticoid and androgenic hormones so that serum cortisol concentration in the animals with IUGR syndrome was 1.58 times lower as compared to those with normal gestation (5.34 ± 0.49 versus 8.46 ± 3.29 nmol/l, $p < 0.05$).

Hormonal and biochemical blood parameters in Red-motley cows at different gestation periods and at intrauterine growth retardation of embryo and fetus (IUGR)
($X \pm x$, Agrotekh-Garant LLC Nashchekino, Annino Region, Voronezh Province, 2013)

Parameter	Days 38-40		Days 60-65		Days 110-115		Days 230-240	
	norm (n = 5)	IUGR (n = 6)	norm (n = 8)	IUGR (n = 10)	norm (n = 5)	IUGR (n = 6)	norm (n = 7)	IUGR (n = 6)
Progesterone, nmol/l	19.2 ± 1.7	$8.0 \pm 0.9^*$	24.9 ± 1.6	$18.7 \pm 1.8^*$	7.48 ± 1.19	7.59 ± 0.51	9.13 ± 0.61	$15.50 \pm 1.20^*$
Testosterone, nmol/l	1.54 ± 0.11	1.48 ± 0.08	1.57 ± 0.12	1.41 ± 0.05	1.33 ± 0.06	1.42 ± 0.12	2.28 ± 0.38	$1.67 \pm 0.10^*$
Estradiol-17 β , nmol/l	0.28 ± 0.02	0.29 ± 0.02	0.49 ± 0.04	$0.23 \pm 0.01^*$	0.33 ± 0.02	$0.44 \pm 0.05^*$	0.75 ± 0.05	$0.52 \pm 0.02^*$
DHEAS, μ g/ml	0.11 ± 0.01	0.13 ± 0.01	0.19 ± 0.01	$0.13 \pm 0.01^*$	0.14 ± 0.01	0.16 ± 0.02	0.33 ± 0.02	$0.25 \pm 0.01^*$
Cortisol, nmol/l	8.46 ± 3.29	$5.34 \pm 0.49^*$	15.70 ± 1.22	$3.44 \pm 0.32^*$	2.46 ± 0.20	2.72 ± 0.19	17.73 ± 1.23	$2.95 \pm 0.21^*$
Triiodothyronin, nmol/l	2.85 ± 0.23	$3.86 \pm 0.27^*$	7.98 ± 0.64	$2.56 \pm 0.22^*$	4.19 ± 0.26	$5.13 \pm 0.33^*$	7.67 ± 0.46	$3.48 \pm 0.24^*$
Total protein, g/l	80.4 ± 1.6	85.4 ± 3.2	79.7 ± 1.5	78.6 ± 1.6	76.2 ± 1.1	81.0 ± 2.9	81.3 ± 2.9	81.5 ± 3.3
Albumin, %	36.2 ± 3.2	37.4 ± 1.9	38.6 ± 2.0	41.2 ± 2.4	50.1 ± 1.5	46.9 ± 2.1	51.2 ± 0.9	48.9 ± 1.9
α -Globulin, %	13.1 ± 1.1	15.6 ± 1.3	13.4 ± 0.6	13.5 ± 0.6	10.6 ± 0.3	12.4 ± 0.7	9.4 ± 0.6	10.2 ± 0.8
β -Globulin, %	21.6 ± 0.7	21.3 ± 0.8	21.9 ± 0.8	22.4 ± 0.7	20.5 ± 0.2	19.1 ± 1.2	18.5 ± 0.8	19.2 ± 0.6
γ -Globulin, %	29.1 ± 2.4	26.6 ± 1.8	26.1 ± 1.2	$22.9 \pm 1.6^*$	18.8 ± 1.4	21.6 ± 1.5	20.9 ± 0.9	21.7 ± 1.5
Total Ig, g/l	32.5 ± 2.4	$26.8 \pm 1.8^*$	28.6 ± 2.1	28.4 ± 2.1	27.0 ± 1.4	$32.6 \pm 1.9^*$	34.9 ± 2.9	$29.4 \pm 2.4^*$
Urea, nmol/l	2.98 ± 0.26	3.29 ± 0.38	3.54 ± 0.40	3.70 ± 0.35	2.40 ± 0.28	2.64 ± 0.20	2.27 ± 0.26	3.28 ± 0.23
Creatinine, mcmol/l	84.0 ± 6.7	95.8 ± 5.9	93.3 ± 6.7	90.5 ± 3.0	92.4 ± 4.6	92.5 ± 3.8	101.0 ± 3.5	102.5 ± 4.4
Glucose, mmol/l	3.72 ± 0.31	4.27 ± 0.21	4.15 ± 0.29	3.89 ± 0.19	2.98 ± 0.22	3.04 ± 0.21	3.24 ± 0.11	3.37 ± 0.19
Lactate, mmol/l	1.24 ± 0.07	1.28 ± 0.05	1.25 ± 0.04	1.20 ± 0.04	1.10 ± 0.02	1.12 ± 0.02	0.85 ± 0.04	0.94 ± 0.09
Pyruvate, mcmol/l	73.6 ± 3.3	61.5 ± 4.5	72.1 ± 6.4	74.4 ± 6.7	117.4 ± 16.1	117.3 ± 14.9	211.0 ± 10.7	198.0 ± 12.6
Vitamin A, mcmol/l	1.58 ± 0.11	1.74 ± 0.29	1.34 ± 0.22	1.60 ± 0.27	1.30 ± 0.15	1.20 ± 0.11	1.31 ± 0.09	1.04 ± 0.11
Vitamin E, mcmol/l	37.8 ± 3.1	31.9 ± 3.4	33.9 ± 4.6	34.9 ± 3.6	25.3 ± 2.3	25.8 ± 2.8	29.4 ± 2.7	28.4 ± 2.0
Vitamin C, mcmol/l	22.2 ± 3.4	$12.4 \pm 2.7^*$	19.6 ± 1.1	$11.2 \pm 0.9^*$	9.7 ± 0.7	$17.2 \pm 1.3^*$	15.1 ± 1.3	$7.4 \pm 0.6^*$
ALP, U/l	105.0 ± 8.5	112.3 ± 12.5	146.6 ± 17.2	120.4 ± 8.9	109.8 ± 7.6	96.8 ± 10.9	83.7 ± 7.2	62.8 ± 5.3
AST, U/l	65.6 ± 5.3	66.1 ± 6.1	64.5 ± 4.6	68.6 ± 4.2	70.3 ± 3.9	59.6 ± 2.2	53.1 ± 3.4	57.8 ± 5.3
ALT, U/l	24.0 ± 2.3	21.2 ± 1.7	20.2 ± 1.1	24.5 ± 1.0	25.4 ± 1.9	26.3 ± 2.9	19.0 ± 0.6	20.9 ± 1.9
GGT, U/L	16.3 ± 1.1	$19.9 \pm 1.1^*$	15.5 ± 0.6	$20.7 \pm 2.1^*$	16.8 ± 1.3	$18.2 \pm 1.4^*$	10.0 ± 0.3	$15.4 \pm 1.2^*$
Phosphorus, mcmol/l	2.07 ± 0.12	$2.63 \pm 0.14^*$	2.10 ± 0.13	2.20 ± 0.13	2.03 ± 0.14	2.40 ± 0.21	2.29 ± 0.07	2.18 ± 0.09
NOx, mcmol/l	102.7 ± 8.3	112.7 ± 10.3	132.4 ± 10.8	$100.7 \pm 8.7^*$	108.5 ± 3.7	$129.1 \pm 5.2^*$	87.2 ± 6.8	$102.3 \pm 7.3^*$
MWP, standard units	0.43 ± 0.04	$0.57 \pm 0.03^*$	0.51 ± 0.03	$0.71 \pm 0.04^*$	0.31 ± 0.03	$0.52 \pm 0.08^*$	0.23 ± 0.02	$0.32 \pm 0.02^*$

Note. DHEAS — dehydroepiandrosterone sulfate, ALP — alkaline phosphatase, AST — aspartate aminotransferase, ALT — alanine aminotransferase, GGT — γ -glytamil transferase, NOx — total nitric oxide stable metabolites, MWP — middle molecular weight peptides.

* $p < 0.05-0.001$ as compared to similar parameters in the cows in physiological gestation.

It is believed that reduced corticosteroid production may manifest in hypothalamic-pituitary dysfunction in relation to the increment of luteinizing hormone [19] which performs a luteotropic function in pregnant animals.

The transition from embryo to the fetal stage (days 60-65) in physiologi-

cal gestation was followed by the increased functional activity of all endocrine glands, as evidenced by the increase in the serum levels of progesterone by 29.7 % ($p < 0.05$), estradiol-17 β by 75.0 % ($p < 0.001$), cortisol by 85.6 %, DHEAS by 72.7 % ($p < 0.001$), and 2.80-fold rise in triiodothyronine level ($p < 0.001$). In IUGR cows progesterone concentrations increased 2.33 times ($p < 0.001$) but remained 33.2 % ($p < 0.05$) lower compared to that of animals in physiological gestation. The levels of other hormones responsible for the synthesis of proteins, bone tissue formation in the fetus and proliferative changes in the tissues of the uterus, decreased compared to baseline values as follows: testosterone by 4.7 %, estradiol-17 β by 20.7 %, cortisol by 35.6 % ($p < 0.05$), triiodothyronine by 33.7 % ($p < 0.05$). As compared to the animals in normal gestation, serum concentrations of testosterone, DHEAS, estradiol β were lower by 10.2 %, 31.6 % ($p < 0.001$), and 53.1 % ($p < 0.001$), respectively, and cortisol and of triiodothyronine levels declined 4.56 times ($p < 0.001$) and 3.11 times ($p < 0.001$), respectively.

In cows with IUGR, immunotrophic interaction of the growing embryo and its mother proceeded against the reduction of serum immunoglobulins by 17.5 % ($p < 0.05$) and increased middle molecular weight peptides by 32.6 % ($p < 0.001$) compared to physiological gestation. Increased concentrations of serum middle molecular weight peptides, on the one hand, indicates activation of serum and tissue protein proteolysis, on the other hand it is the evidence of detoxification processes violation. As molecular analogues of regulatory peptides, MWP are able to block cell membrane receptors, decrease albumin transport capability, and break many metabolic processes in pregnant animals [20].

GGT is known to provide energy dependent amino acid transport into cells thus regulating the serum level of total protein and its fractions. We associate the GGT activity increase by 22.1-33.5 % ($p < 0.05$) in IUGR cows compared to physiological gestation with the enzyme involvement in the processes of detoxification and amino acid pool stabilization, the imbalance of the latter being the most important pathogenetic factor of endogenous intoxication [21].

At placentation phase (60-65 days), IUGR cows demonstrated reduced vitamin C and NO $_x$ levels compared to the animals in physiological gestation (by 42.9 and 23.9 %, respectively, $p < 0.001$). Low vitamin C were found in the IUGR cows at days 38-40 — 12.4 ± 2.7 versus 22.2 ± 3.4 nmol/l ($p < 0.001$) in normal embryo growth. Since vitamin C provides antioxidant embryo protection [22] and is involved in the formation of fetal connective tissue [23, 24], its reduced levels in pregnant animals should be regarded as a very unfavorable factor for embryo and fetus development.

Nitric oxide is the key regulator of placental angiogenesis and placental-fetal blood flow [3, 25-27]. Reduced nitric oxide synthesis during pregnancy is associated with delayed formation of placental blood flow, violation of the transfer of nutrients and oxygen, which results in embryo and fetus growth retardation.

At the stage of fetoplacental complex formation (days 110-115), the differences in the hormonal status in IUGR cows and in physiological gestation were less pronounced (see Table). We attribute this to a decline in the endocrine gland hormone synthesizing function necessary for normal growth of a cow fetus [8, 10] which was not observed in IUGR cows. In this period, a start of compensatory mechanisms was registered in the IUGR cows: serum total immunoglobulin increased by 14.8 % ($p < 0.05$), vitamin C increased by 53.6 % ($p < 0.001$), stable nitric oxide metabolites increased by 28.2 % ($p < 0.05$) compared to the previous period. The levels of these substances exceeded that in the cows with normal fetus development by 20.7 % ($p < 0.05$), 77.3 % ($p < 0.001$), and 19.0 % ($p < 0.05$), respectively. However, the expected reduction in endogenous intoxication indicators

was not observed. The blood levels of middle molecular weight peptides in these animals remained relatively high, being 67.7 % greater compared to physiological gestation ($p < 0.05$). GGT activity was also 8.3 % greater ($p < 0.05$).

At the final gestation (days 230-240), hormone producing reserves of endocrine glands, fetoplacental complex and hormone synthesizing function were much higher in the cows with normal embryo development than in the animals with IUGR diagnosed at days 38-40 (see Table). In the latter ones, the levels of main serum hormones that form the parturition dominant — cortisol, estradiol, testosterone and DHEAS were 6.01 times ($p < 0.001$), 1.44 times ($p < 0.001$), 1.37 times, and 1.32 times ($p < 0.001$) lower, respectively. In contrast, in these cows the progesterone which blocks the uterine contractility and prolongs gestation was 1.70 times higher ($p < 0.001$). Progesterone-estradiol ratio was 29.8 in IUGR cows versus 12.2 in cows in physiological gestation. Furthermore, serum triiodothyronine levels reduced 2.20 times ($p < 0.001$) in the IUGR cows, which was followed by a deceleration in all metabolic processes. During this period, the IUGR cows demonstrated a decrease in immunoglobulins (15.8 % lower, $p < 0.05$) and vitamin C levels (2 times lower, $p < 0.01$), while middle molecular weight peptides were 39.1 % higher ($p < 0.01$), stable nitric oxide metabolites were 17.3 % higher ($p < 0.05$), and GGT activity increased by 54.0 % ($p < 0.01$). Activated synthesis of placental nitric oxide which has muscle relaxant effects, and progesterone is probably targeted at the uterine contractility restriction to prolong the gestation in IUGR cows.

Thus, realization of the genetic program of fetus formation and development is largely determined by the synthesis and metabolism of sex, corticosteroid and thyroid hormones which are specific regulators of biochemical and biophysical processes in the organisms of mother cow and the fetus. The key point in the growth retardation of embryo and fetus (IUGR) syndrome is the violation of embryo nutrition at implantation and early placentation associated with incomplete secretory endometrium transformation and a delay in the formation of placental-fetal blood flow which is due to an imbalance of sex steroids and decrease in the nitric oxide synthesis. Endogenous intoxication should be considered as one of the determining factors of functional insufficiency in biological mother-embryo-fetus system. The revealed pathogenetic mechanisms causing violations in formation, growth and development of embryo and fetus may be the basis for the development of effective strategies for IUGR prevention and therapy in farm animals.

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