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GENETIC CAUSES OF BIRTH OF IMPAIRED CALVES AND REPRODUCTION DYSFUNCTION OF A DAIRY HERD

Vladimir Trukhachev, Serhii Oliinyk, Nikolai Zlydnev, Vitaly Morozov, Nikolai Belugin, Tatiana Aleksandrova, Elena Zorina

Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia

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Abstract

Modern genotypes of dairy cattle created on the base of the Holstein breed are characterized by improved external conformation performance relatively to the milk-type expression of an animal and increased in comparison to the first mother rocks, by 25-50% by the milk production parameters, annual yield of the best cows in commodity farms can often reach 7500-9000 kg of milk. However, the uncontrolled use of bulls-improvers can often lead to disruption of the process of reproduction of the herd and the birth of weakened and unviable calves.

Analysis of the causes of the disturbance of the normal reproduction process in the dairy herd of black and white cattle, created by absorbing crossing with the Holstein bulls, showed that the cause of the birth of a weakened youngster with signs of deformity (internal organ dystopia, aplasia and hypoplasia of limbs, absence of lumbar vertebrae, eye pathology, atresia of anal verge) and clinical signs of a disturbance of the function of the gastrointestinal tract (diarrhea, refusal of feed, lethargy, oppression), dehydration (dryness and loss of skin elasticity, retraction of an eyeball), thickening of the joints was the use of the selection of parental pairs of seed bull with a genetic anomaly HH1C. In this case, the genetic analysis of the brood stock according to 12 types of haplotypes, associated with impaired fertility, and to 31 types of monogenic diseases showed that the reason of the birth of impaired calves was connected with genetic anomalies CVM and BLAD only for 6.9% of the breeding stock.

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Key words: genetic anomalies, pasture stock raising, dairy cattle.

Introduction

Intensive use of the world breed gene pool of cattle and reproduction biotechnologies (artificial insemination, embryo transplantation) allowed significantly increasing the genetic potential of animal productivity. In the last 30 years (the end of the twentieth century - the beginning of the 21st century), the number of the black-and-white Holstein breed and genotypes created with its use in Russia and all over the world took the leading place among the cattle breeds of the dairy direction of productivity. Thanks to the import of pedigree material from the USA and Canada to Europe, as well as to other continents and the intensive use of Holstein breeds in black- and- white cattle in a number of countries, the yield of cows increased sharply, by some herds by 25-50% (Bukarov, N. G., 2004; Kuznetsov VM, 2001). This is due to their high genetic potential of dairy productivity, early maturity, ability to intensive milk yield, fitness for machine milking and other valuable qualities of animals, which was especially evident in the ICAR member countries (Bagirov V.A., 2009; Yanchukov, I. N., 2012; The Global standard for livestock data: 01 - National Milk Production, 2018). At the same time, the widespread use of a limited contingent of bulls-producers led to the fact that in different countries cases of the birth of calves with various phenotypic anomalies, which, according to genetic analysis, had a hereditary basis due to mutations of the genes began to be recorded.

Modern methods of large-scale selection based on the use of artificial insemination make it possible to obtain tens of thousands of offspring from each bull-manufacturer, which considerably shortens the time for the introduction of valuable gene complexes in the selection process, on the one hand, the real danger of genetic erosion - depletion or narrowing of the gene pool and also if breed leaders are carriers of a harmful mutation, this mutation can reach in the short term millions of heads and needs for its elimination long time and huge amounts of money, on the other hand (Glazko,V. I., 2009; Ernst, L. K., 2009).

To prevent these negative phenomena in many countries with developed animal husbandry, national genetic monitoring programs operate including as a mandatory element genetic examination of animals for the presence of hereditary genetic abnormalities. In Russia since 2016 the organization of regular monitoring of genetic defects of bulls-manufacturers has been introduced with the introduction of appropriate recommendations and instructions in the breeding service (Department for Quality Control and Standardization of Genetic Material and Preparations Used in the Reproduction of Animals of the FGU «VGNKI», 2018). And although the issues of legal liability to farms for economic damage caused by genetic diseases as a result of the sale and purchase of a defective seed are still not completely clear, farmers and dairy cattle owners already prefer to purchase a bovine seed that is safe for known genetic abnormalities (Turbina, I.S. and others, 2004).

One of the negative consequences of genetic abnormalities for dairy cattle breeding is deterioration of reproductive capacity of the breeding stock and a decrease in the viability of newborn young animals. Therefore

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the possibility of obtaining a healthy gamete in a female that is able to give normal development to the embryo and fetus, as well as to ensure the normal birth and the survival rate of the offspring depends on the fertility of the two participants in the parent couple. The Holstein cattle have already identified 17 haplotypes that adversely affect fertility, while Russia is actively studying the spread of five haplotypes, the most common among Holstein dairy cattle. Haplotype is a sequence of nucleotides located in one chromosome and transmitted from parents to children without changes. If a mutation occurs inside the haplotype, a new haplotype is formed. A descendant may receive from the ancestor only one copy of the haplotype. For the manifestation of the negative impact of the haplotype, the descendant should receive two identical copies of the DNA site, one from the father and one from the mother. According to the adopted nomenclature, the name of the haplotype consists of two Latin letters and a serial number. The first letter stands for the breed, the second letter stands for H from the haplotype. The six haplotypes - HH0, HH1, HH2, HH3, HH4, HH5 - were most studied in Holstein. All embryos that are homozygous for some haplotype of the above are not viable (Zinovieva, N.A., etc., 2012, 2015; Official website of the genetic company "My Gene", 2018, VanRaden, PM at all, 2011).

Thus, in Russian dairy cattle breeding, there is also a practice of expanding the use of molecular genetics methods to improve the efficiency of herd management (Barsukova, O.E., 2012; Dementieva, N.V., 2014).

In this paper the results and analysis of the causes of the violation of the normal reproduction process in the dairy herd of black-and-white cattle with using bulls of the Holstein breed are presented. Therefore the task of our studies was to determine the causes of pathology in calves in prenatal and postnatal periods by complex measures of etiology.

Clarification of the reasons for the decline in the reproduction of the herd is an important component of the organization of an effective system of grazing livestock.

One of the indicators influencing the realization of the genetic potential of farm animals is the quality of the feed. The herbage of the farm pasture, which was used for the rearing of young animals, consisted of legume-cereals, namely clover meadow, alfalfa, awnless brome and wheatgrass, with the ratio of cereal and leguminous grasses being 4: 1. The task of the study was also to develop a methodology for remote assessment of the nutritional value of the farm pasture areas.

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Materials and methods of research

Research work was carried out on a dairy farm of black-and-white cattle in the Stavropol Territory of the Russian Federation. Milk productivity on an average in the herd with a total number of cattle counts 100 cows, averaged 7000 thousand kg of milk per year for 1 cow, milk fat content in milk was 3.85%, milk protein - 3.15%, inter-interval was within the limits of 385-405 days. However, in the period 2016-2017, on the dairy farm there were observed cases of the birth of weak young, some calves showed signs of deformity (internal organs dystopia, aplasia and hypoplasia of limbs, absence of lumbar vertebrae, eye pathology, anal atresia, etc.). The majority of the newborn calves, starting from the second day of their life, had clinical signs of a disturbance of the function of the gastrointestinal tract (diarrhea, rejection of feed, lethargy, depression), dehydration (dryness and loss of skin elasticity, eyelid drooping), joint thickening.

To determine the causes of the birth of young animals with an abnormal manifestation of phenotypic traits, microbiological studies of blood in mother cows and their offspring were conducted to confirm the infectious nature of the diseases - leptospirosis, brucellosis, Escherichia coli, salmonella.

For in-depth study of the causes of the birth of a weakened youngster, genetic studies were conducted on the following parameters: leukocyte adhesion deficiency - BLAD; deficiency of uridine monophosphate synthase-DUMPS; complex vice of the spine - CVM; citrullineemia - BC; brachispine - BY; deficiency of factor XI (eleven) blood - FXID; axonopathy - DS; bull subfertility - BMS; Chediak-Higashi syndrome - CHS; congenital muscular dystonia of type 1 - CMD1; congenital muscular dystonia of type 2 - CMD2; Curved tail syndrome - CTS; dwarfism of the "bulldog" type - BD; bullous epidermolysis - EB; deficiency of factor VIII (eight), hemophilia A - FVIIID; idiopathic congenital mega-esophagus - ICM; a-mannosidosis - a-MAN; β-mannosidosis - b-MAN; mucopolysaccharidosis - MPSIIIB; maple syrup disease (valinoleucinuria) - MSU; Syndactyly, mule hoof - Mulefoot; neuronal ceroid lipofuscinosis - NCL; osteopetrosis - OS; syndrome of a swollen calf - PCS; congenital pseudomonia - PMT; congenital erythropoietic protoporphyria -PT; arachnomelia and arthrogryposis syndrome - SAA; spinal demyelination - SDM; spinal muscular atrophy - SMA; thrombopathy - TP; Weaver Syndrome - Weaver.

Genetic studies were carried out using a dermal pluck of cows as a biological material, from which genomic DNA was isolated using Qiagen columns, the Netherlands called QIAamp DNA Blood Mini Kit based on membrane-silicon technology in accordance with the manufacturer's protocol. The carrier status of recessive monogenic cattle diseases was determined using the polymerase chain reaction method with further electrophoretic analysis of the amplicon, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

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method, monogenic diseases, or the method of sequencing the target regions of the cattle genome, the determination of haplotypes. Polymerase chain reaction with further electrophoretic analysis of amplicon was used to determine the status of the diseases: FXID, BY, HH5, HCD.

Polymerase chain reaction method with further electrophoretic analysis of the amplicon: in this procedure, polymerase chain reaction was carried out in a volume of 10 µl containing 10 ng DNA, 2 µl PCR buffer 5X MasCFETaqMIX (Dialat, Moscow) and 0.4 µl primer (concentration 2, 5 pmol/100 µl). A polymerase chain reaction was carried out on a C1000 ™ Thermal Cycler (BioRad, USA). The PCR products were separated in a 2% agarose gel, visualized in an ultraviolet transilluminator and detected with a gel documentation system. The determination of carrier status was established on the basis of the identification of bands in the agarose gel that correspond to DNA fragments of a certain length.

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP): Within the framework of this procedure, a polymerase chain reaction was carried out in a volume of 10 µl containing 10 ng DNA, 2 µl of 5X MasCFETaqMIX PCR buffer (Dialat, Moscow) and 0.4 µl of primer (concentration 2.5 pmol/ 100 µl). A polymerase chain reaction was carried out on a C1000 ™ Thermal Cycler (BioRad, USA). Next, 10 µl of the restriction mixture containing 0.7 µl restriction endonuclease, 1.3 ml of 10 × buffer for the selected restriction endonuclease and 8 µl of water were added to 10 µl of the amplicon obtained. The resulting mixture was incubated according to the protocol of the manufacturer of restriction endonucleases on the C1000 ™ Thermal Cycler (BioRad, USA). The incubated restriction mixture was separated in a 4% agarose gel, visualized in an ultraviolet transilluminator and detected with a gel documentation system.

Table 1. Explanation of the symbols for the carriage of monogenic diseases

№	The name of a monogenic disease	Abbreviation	Not carrier	Carrier	Carrier in homozygous state
1	Leukocyte adhesion deficiency	BLAD	TL	BL	-
2	Deficiency of uridine monophosphate synthase	DUMPS	TD	DP	-
3	Complex vice of the spine	CVM	TV	CV	-
4	Citrullineemia	BC	CNF	CNC	-
5	Brachispine	BY	TY	BY	-
6	Deficiency of factor XI (eleven) blood	FXID	XIF	XIC	XIS
7	Axonopathy	DS	DSF	DSC	-
8	Bull subfertility	BMS	MSC	MSF	MSS
9	Chediak-Higashi syndrome	CHS	CHSF	CHSC	CHSS
10	Congenital muscular dystonia of type 1	CMD1	MD1F	MD1C	MD1S
11	Congenital muscular dystonia of type 2	CMD2	MD2F	MD2C	MD2S
12	Curved tail syndrome	CTS	CTF	CTC	CTS
13	Dwarfism of the "bulldog" type	BD	BDF	BDC	-
14	Bullous epidermolysis	EB	EBF	EBC	EBS
15	Deficiency of factor VIII (eight), hemophilia A	FVIIID	VIIIF	VIIIC	VIIIS
16	Idiopathic congenital mega-esophagus	ICM	ICMF	ICMC	ICMA
17	a-mannosidosis	a-MAN	aMANF	aMANC	-
18	β-mannosidosis	b-MAN	bMANF	bMANC	-
19	Mucopolysaccharidosis	MPSIIIB	SIIIBF	SIIIBC	SIIIBS
20	Maple syrup disease (valinoleucinuria)	MSU	MSDF	MSDC	MSDS
21	Syndactyly, mule hoof	Mulefoot	MFF	MFC	MFS
22	Neuronal ceroid lipofuscinosis	NCL	NCLF	NCLC	NCLS
23	Osteopetrosis	OS	OSF	OSC	-
24	Syndrome of a swollen calf	PCS	PCSF	PCSC	-
25	Congenital pseudomonia	PMT	PMTF	PMTC	PMTS
26	Congenital erythropoietic protoporphyria	PT	PTF	PTC	PTS
27	Arachnomelia and arthrogryposis syndrome	SAA	SAAF	SAAC	-
28	Spinal demyelination	SDM	DMF	DMC	-
29	Spinal muscular atrophy	SMA	MAF	MAC	-
30	Thrombopathy	TP	TPF	TPC	TPS
31	Weaver (Weaver Syndrome)	Weaver	WF	WC	WS

The determination of carrier status was established on the basis of the identification of bands in the agarose gel that correspond to DNA fragments of a certain length.

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Table 2. Haplotypes associated with impaired fertility

№	Название гаплотипа	Abbreviation	Not carrier	Carrier	Carrier in homozygous state
1	Holstein haplotype 1	HH1	HH1F	HH1C	-
2	Holstein haplotype 2	HH2	HH2F	HH2C	-
3	Holstein haplotype 3	HH3	HH3F	HH3C	-
4	Holstein haplotype 4	HH4	HH4F	HH4C	-
5	Holstein haplotype 5	HH5	HH5F	HH5C	-
6	Holstein haplotype, associated with cholesterol deficiency	HCD	CDF	CDC	CDS
7	Ayrshire haplotype 1	AH1	AH1F	AH1C	-
8	Haplotype 1 brown Swiss breed	BH1	BH1F	BH1C	-
9	Haplotype 2 brown Swiss breed	BH2	BH2F	BH2C	-
10	Jersey haplotype 1	JH1	JH1F	JH1C	-
11	Jersey haplotype 2	JH2	JH2F	JH2C	-
12	Montbeliard haplotype 2	MH2	MH2F	MH2C	-

The remote method for assessing the nutritional value of the farm pastures was carried out using a multi-rotor unmanned aerial vehicle and Canon M10 camera. Taking into account the fact that the characteristic feature of vegetation and its state is the spectral reflectivity characterized by large differences in the reflection of radiation of different wavelengths, the index-vegetation index NDVI was used to assess the quality of pasture feeds. Chemical studies of feeds were carried out according to standard methods.

Research results and discussion

Based on the results of microbiological research on the main types of infectious diseases, it was established that there are no infectious causes for the manifestation of phenotypic abnormalities in newborn calves.

Analysis of technological conditions of feeding and content showed that the level of feeding of dairy cattle corresponds to the norms for different sex and age groups. Thus, the cows were excluded from parasitic and infectious causes of manifestations of these abnormalities in newborn young animals.

Conducting veterinary clinical diagnosis in calves, showed the manifestation of the following pathologies, which was observed in calves, regardless of the age of mothers, whether they be heifers or cows (Table 3).

Table 3. Results of the manifestation of pathology in calves

Pathology name	Number of calves, heads	Percentage of total amount, %
Osteodystrophy	5	20.0
Fetal hypotrophy	3	12.0
Anomaly of the dental occlusion, softness of the teeth	2	8.0
Pathology of the eye, blindness	4	16.0
Nervous fever	5	20.0
Absence of a sucking reflex	2	8.0
Aquarius of the abdominal cavity	1	4.0
Non-disclosure of lungs, atelectasis	3	12.0
Atresia of the anal opening	2	8.0
Total	25	100.0

At the time of the survey, calves aged from one to 15 days old were in the maternity ward. At the same time, all showed signs of diarrhea, the feces were mostly semi-fluid, and in 5 calves with a dense mucus admixture, twelve calves showed thickening of the wrist joints, three of the calves examined had eyelids, some calves showed umbilical inflammation, six calves with clinical signs of toxic dyspepsia (apathy, lack of response to external irritants, dryness and reduced skin elasticity, ruffiness and dullness of the coat, around the tail and pelvic limbs are stained with liquid whitish- yellow color excreta, westing of the eyeball), incisors in most calves were shattered.

A diagnostic autopsy of one fallen bull-calf was carried out 14 days old.

In pathoanatomical research, the following changes were found: dryness and loss of skin elasticity, eyelid gland westing, dryness of subcutaneous tissue; depletion, lack of fat in the subcutaneous tissue; catarrhal ulcer inflammation of the abomasum, the presence of phytobezoar from straw in the rumen, focal flushing of the rumen wall, the presence of milk clots in the rumen and a dirty gray semisolid mass; hyperkeratosis of the papillae of the book; acute muco-catarrhal enterocolitis; congestive hyperemia and albuminous dystrophy of the liver; serous lymphadenitis of mesenteric (mesenteric) lymph nodes; focal atelectasis of the lungs, hypoplasia of the red bone marrow; catarrhal cholecystitis, dystrophy and focal necrosis in the biceps ham muscles located symmetrically.

On the basis of the autopsy of the fallen calf, pathologoanatomical changes characteristic of toxic dyspepsia, which developed against a background of hypotrophy, were found.

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In a more detailed analysis of the state of reproduction of a herd of cows and heifers, it was established that artificial insemination was carried out by the seed of a single bull producer, who, according to the results of genetic studies, established the genetic anomaly of HH1C (Table 4). In this case, the genetic analysis of the broodstock in 12 types of haplotypes associated with impaired fertility and 31 types of monogenic diseases showed that only 3.4% of the breeding stock caused the appearance of genetic anomalies of CVM and BLAD (Table 4).

Table 4. Monitoring results for carriage of monogenic diseases

№	Status	Manifestation of genetic anomalies	Breed	Number of heads in the group	Age, years	Research results
1	Cow	CV	Black-and-white	1	7,0	HH1F*, HH2F, HH3F*, HH4F*, HH5F*, CDF*, AH1F, BH1F, BH2F, JH1F*, JH2F, MH2F*, TL, TD, **CV**, CNF, TY, XIF, DSF, MSF, CHSF, MD1F, MD2F, CTF, BDF, EBF, VIIIF, ICMF, aMANF, bMANF, SIIIBF, MSDF, MFF, WF, NCLF, OSF, PCSF, PMTF, PTF, SAAF, DMF, MAF, TPF
2	Cow	BL	Black-and-white	1	5,0	HH1F*, HH2F, HH3F*, HH4F*, HH5F*, CDF*, AH1F, BH1F, BH2F, JH1F*, JH2F, MH2F*, **BL**, TD, TV, CNF, TY, XIF, DSF, MSF, CHSF, MD1F, MD2F, CTF, BDF, EBF, VIIIF, ICMF, aMANF, bMANF, SIIIBF, MSDF, MFF, WF, NCLF, OSF, PCSF, PMTF, PTF, SAAF, DMF, MAF, TPF
3	Cow	Absence	Black-and-white	27	6,0	HH1F*, HH2F, HH3F*, HH4F*, HH5F*, CDF*, AH1F, BH1F, BH2F, JH1F*, JH2F, MH2F*, TL, TD, TV, CNF, TY, XIF, DSF, MSF, CHSF, MD1F, MD2F, CTF, BDF, EBF, VIIIF, ICMF, aMANF, bMANF, SIIIBF, MSDF, MFF, WF, NCLF, OSF, PCSF, PMTF, PTF, SAAF, DMF, MAF, TPF
4	Bull-manufacturer	HH1C*	Holstein	1	9,0	**HH1C***, HH2F, HH3F*, HH4F*, HH5F*, CDF*, AH1F, BH1F, BH2F, JH1F*, JH2F, MH2F*, TL, TD, TV, CNF, TY, XIF, DSF, MSF, CHSF, MD1F, MD2F, CTF, BDF, EBF, VIIIF, ICMF, aMANF, bMANF, SIIIBF, MSDF, MFF, WF, NCLF, OSF, PCSF, PMTF, PTF, SAAF, DMF, MAF, TPF

* - the status is confirmed by testing of casual mutation.

In our study, the results of remote assessment of pasture land with the use of unmanned aerial vehicles NDVI was in the range of 0.55 to 0.65, an average of 0.60±0.02. Chemical analyses of the feed showed that the higher vegetation index, the more nutritious the food. The crude protein content in air-dry substance was within 8.9-10.2%, humidity 3.4-3.9%, crude fiber 28.6-30.2%, raw fat – 2.5-2.8%, crude ash 6.9 to 7.1 percent. It is established that the cultivation of highly productive farm animals on the grazing areas is advantageously carried out at a value not lower than NDVI of 0.55.

Conclusion

The results of screening studies show that one of the reasons for the manifestation of phenotypic and clinical abnormalities in newborn young animals may be the presence of the genetic anomaly of HH1C in the father-bull, while the broodstock remains safe on the indicated trait.

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Data about the authors:

Vladimir Trukhachev. Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail: rector@stgau.ru,

Serhii Oliinyk. Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail: soliynik60@gmail.com,

Nikolai Zlydnev, Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail: akusherstvo.nikitin@yandex.ru

Vitaly Morozov, Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail:supermoroz@mail.ru

Nikolai Belugin, Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia.

Tatiana Aleksandrova, Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail: alexandrova_026@inbox.ru,

Elena Zorina. Federal State Budgetary Education Institution of Higher Education «Stavropol state agrarian university», Russia. E-mail: zeb26@mail.ru