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The use of whole genome amplification for genomic evaluation of bovine embryos

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The integration of high technologies into livestock production has been actively occurring in the last decade in the countries with a developed animal breeding. First of all, we are talking about reproductive technologies (IVF) and genomic technologies (general genomic evaluation of animal and genomic evaluation of breeding value). Combining reproductive and genomic technologies is a promising approach that allows receiving high-quality breeding cattle in the shortest possible time. The basis of the proposed technology for accelerated reproduction of high-value breeding cattle is to obtain information about the genome of the embryo for genomic evaluation. The amount of genetic material that can be obtained for research is extremely limited, as it is necessary to preserve the viability of the embryo. The stage of the whole genome amplification was introduced to obtain a high quality of genetic material in a sufficient quantity. The main purpose of this work is to assess the possibility of using embryo biopsy specimens (bsp) for embryo genotyping using microarray chips and predicting the carrier status of lethal haplotypes at the embryo stage. We obtained 100 cattle embryos, of which 78 biopsy specimens were taken to analysis. For the biopsies obtained we performed the whole genome amplification. The quality and quantity of DNA for all the 78 samples after the whole genome amplification were satisfactory for further genotyping. The quality of the performed genotyping was satisfactory and allowed the assessment of lethal haplotype carriers (determining the sex of the animal and identification of the carrier status for seven Holstein lethal haplotypes). We tested 78 embryos. From the genotyping analysis, there was detected one carrier status for three lethal haplotypes, HH0 (Brachyspina), HH5, and HCD. The carrier status of HH0 and HH5 was confirmed by testing the casual mutation using PCR analysis. The carrier status for HCD has not been confirmed by casual mutation analysis. The situation in which an animal is an HCD carrier, but not the carrier of a casual mutation, can be explained. The putative ancestor of the haplotype is the bull HOCAN000000334489 WILLOWHOLME MARK ANTHONY (year of birth is 1975), but a casual mutation associated with this disease has arisen only in his descendant HOCAN000005457798 MAUGHLIN STORM (year of birth is 1991). The results obtained confirm the importance of testing the casual mutation in the animals that are carriers of lethal haplotypes according to the genotyping data.

Key words: cattle; dairy direction; breeding; genomic evaluation; breeding animals.

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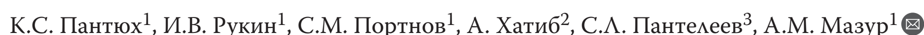
Применение полногеномной амплификации для генетической оценки эмбрионов коров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В странах с развитым животноводством в последнее десятилетие активно происходит интеграция наукоемких технологий в племенное животноводство. В первую очередь речь идет о репродуктивных технологиях (ЭКО) и геномных технологиях (оценка носительства летальных гаплотипов и геномная оценка племенной ценности). Комбинирование репродуктивных и геномных технологий – перспективный подход, который позволит получать племенной скот высокого качества в кратчайшие сроки. В основе предлагаемой технологии ускоренного воспроизводства высокоценного племенного скота лежит получение информации о геноме эмбриона для проведения геномной оценки. Так как необходимо сохранить эмбрион живым, то количество генетического материала, который можно получить для исследований, крайне ограничено. Чтобы получить ДНК высокого качества и в достаточном количестве, при прове-

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дении генотипирования на чипах вводится этап полногеномной амплификации ДНК. Основной целью работы была оценка возможности использования биоптата (бп) эмбрионов для генотипирования и предсказания носительства летальных гаплотипов на основе результатов генотипирования. Нами было получено 100 эмбрионов крупного рогатого скота, из которых удалось взять 78 биоптатов. Полученные биоптаты были использованы для проведения полногеномной амплификации и генотипирования с применением микроматрицы. Качество и количество ДНК после проведения полногеномной амплификации всех 78 образцов были удовлетворительными для дальнейшего генотипирования. Результаты генотипирования позволили провести расчет пола животного и определение статуса носительства семи основных летальных гаплотипов голштинской породы. Из 78 протестированных животных по результатам анализа генотипа были найдены 3 носителя летальных гаплотипов – HH0 (брахиспина), HH5 и HCD. Носительство летальных гаплотипов HH0 и HH5 было подтверждено тестированием мутации, влияющей на потерю фертильности (казуальной) с помощью ПЦР-анализа. Статус носительства гаплотипа HCD после тестирования казуальной мутации не был подтвержден. Отсутствие казуальной мутации HCD у животного-носителя гаплотипа HCD можно объяснить тем, что предположительным родоначальником гаплотипа HCD является бык HOCAN000000334489 WILLOWHOLME MARK ANTHONY (год рождения – 1975), в то время как казуальная мутация, связанная с появлением заболевания, возникла в этом гаплотипе уже у его потомка, быка HOCAN000005457798 MAUGHLIN STORM (год рождения – 1991). Полученные данные подтверждают важность тестирования казуальной мутации у животных-носителей летальных гаплотипов.
Ключевые слова: крупный рогатый скот (КРС); молочное направление; племенное разведение; геномная оценка; племенные животные.

Introduction

In dairy industry, cows are bred to produce a large quantity of milk and dairy products. In the Russian Federation, dairy products belong to the group of food products that are socially very important. In 2008, milk production in the Russian Federation amounted to about 32.3 million tons, and in 2015 milk yield decreased by 4.7 % to 30.8 million tons. The milk production is decreasing while a lot of citizens cannot afford a sufficient amount of milk and dairy products. According to the World Health Organization, the annual minimum level of milk and dairy product consumption is 359 kg per capita; however, this figure is only 249 kg in the Russian Federation. The absence in the Russian Federation of modern animal breeding programs for dairy cattle is the main cause of the current situation.

Milk production can be significantly increased by applying selection programs that are oriented towards increasing milk yield. The response to selection is measured as the annual genetic progress in a population (ΔG). The value of genetic progress in a population depends on the variability of the trait that animals are selected for, the selection intensity, the accuracy of estimated breeding value, and the generation interval. The implementation of genomic evaluation of breeding values can improve three of the four factors that affect the genetic progress in the population (Boicharda et al., 2016). In particular, genomic evaluations can increase the accuracy of estimation of breeding value by 40 % in comparison with the accuracy obtained from the traditionally estimates of the animal parent averages. In addition, generation interval can be reduced two to three times, and selection intensity can be greatly increased due to the ability of choosing candidate young bulls from a relatively larger number of animals (Food and Agriculture Organization of the United Nations, 2007). Consequently, the implementation of genomic evaluation of breeding values increases the annual genetic gain in population three times more than that achieved by traditional progeny testing, and reduces the cost for every unite of the genetic gain by 100-fold (Kuznetsov, 2015).

Another concern in the modern dairy industry is the decrease of cattle fertility. Ignoring fertility traits in selection programs and the intensive selection for increased milk yield for many years has been accompanied by declining the reproductive performance of dairy cattle (Ma et al., 2018). In addition, the decline in female fertility can be explained, in part, by genetic factors. In fact, there are unfavorable genetic correlations between milk yield and fertility. Furthermore, in the past few years, there have been identified many genetic defects that associate with the loss of fertility. These defects are mainly inherited in the autosomal recessive manner and cause the embryonic loss in homozygous state. An approach that was developed by P.M. VanRaden and his colleagues was used to detect most of these genetic defects. The concept of this approach is that lethal recessives can be discovered from haplotypes that are common in the population but are never homozygous in live animals (VanRaden, et al., 2011). These genetic defects have been called "lethal haplotypes". Using this method has resulted in identification of seven lethal haplotypes (HH1, HH2, HH3, HH4, HH5, HCD, and HH0) in Holsteins. Another new lethal haplotype, HH6, is now being tested in the Holstein breed (Fritz et al., 2018). The carrier status of lethal haplotypes is not included in the genomic evaluation of breeding value. Genetic monitoring to identify animal carriers of monogenic diseases and haplotypes is extremely important. The comprehensive information obtained from genetic monitoring of an animal for the carrier status for monogenic diseases, carrier status for lethal haplotypes, allelic composition for milk protein genes and other economically important traits should be included in – as can be called – the animal genetic passport.

The ability of carrying out the genomic evaluation of breeding value for the viable animal embryos and monitoring these embryos for genetic defects would considerably

accelerate the production of high-genetic-merit animals because only the embryos that are non-carriers for monogenetic diseases and have the highest breeding value will be selected and transplanted.

Materials and methods

Embryos production. To produce embryos, 17 Black-and-White bulls were primarily selected to serve as service sires.

One straw of semen was collected from each sire in a volume of 250 µl. The final selection of bulls was performed based on the quality of collected semen, pedigree analysis, and the estimated breeding value of ancestors of the bulls. As a result, 12 sires were selected from three farms: the head center for the reproduction of farm animals (6 sires), "Moskovskoe" for breeding work (3 sires), and "Alta Genetics Russia"(3 sires). Semen of bulls was transported in liquid nitrogen in a Dewar tank into the station where bovine oocytes are collected from donor cows.

Holstein cows from the farm "Permskaya Kraya" were selected to serve as donor cows. The selection was based on the age, high reproductive performance, and production indicators of the cow, taking into account the breeding schemes of the farm. Cows that were excluded were sick cows, cows that showed low levels of activities, cows that were in the period of progressive weight loss after calving, exhausted and obese cows. In order to obtain large numbers of oocytes from each donor cow, an echographic characteristics analysis of the ovarian was performed.

After the aspiration, liquid aspiration was washed using Dulbecco's buffer solution. The search of oocytes was conducted under a binocular loupe. The suitability of oocytes for maturation was visually evaluated. Oocytes that have been remained for the following *in vitro* maturation are those that met the following conditions: viable, evenly surrounded by cumulus cells, a fine-grained ooplasm that evenly fulfills the transparent shell of the oocyte, a homogenous thickness of the transparent shell with a round shape. Selected oocytes of the required quality were set to mature for 22 h in an IVM media. After maturation, oocytes were washed from the IVM media and transferred to the Fertilization Medium. Spermatozoa were washed by centrifugation on a discontinuous 45 : 90 Percoll gradient and prepared for oocytes fertilization *in vitro* (IVF). Oocytes cultivation was performed on a palate incubator under a constant temperature, regulated humidity and gaseous environment. On the 6th day of cultivation, in the incubator, the obtained embryos were evaluated and only high-quality embryos were selected for biopsy. Biopsy was performed at the blastocyst stage using a biopsy needle. 30–50 cells were taken from the trophoblasts of the blastocyst. Cells were counted while they were aspirated into a biopsy pipette. Biopsy specimens in biopsy pipette were released into a 2 µl PBS × 2 buffer. The drop containing the embryonic trophoblast cells was placed at the bottom of the LoBind tube whose bottom was previously prepared with a 2.5 µl drop of PBS × 2 buffer. After the biopsy, embryos were marked using necessary markers to identify them during the next stages of the experiment.

Embryo viability was monitored for 8–24 h prior to cryopreservation. All the pedigree information and the identification number of biopsy were saved for each embryo.

Whole genome amplification and genotyping. The method of isothermal multiple displacement amplification (IMDA) was used for the whole genome amplification (WGA). The whole genome amplification was performed using GenomiPhi V2 DNA amplification kit (Illumina, USA) considering the standard recommendations for it. The whole genome amplification was performed for 78 biopsy specimens. After the whole genome amplification, the quantity of DNA for each sample was measured using NanoDrop ND1000-Technologies-Inc, Wilmington, DE, USA, while the quality of DNA was checked using agarose gel electrophoresis. The DNA concentration was adjusted to 50 ng/µl. For genotyping, 4 µl from each sample was taken, and BovineSNP50 v3 DNA Analysis BeadChip was used considering the instructions provided in the manual protocol for this microarray: Infinium® HD Assay Ultra, Manual Experienced User Card (Part # 11328095 Rev. B, Illumina, USA).

Identification of the carriers of lethal haplotypes. For the subsequent analysis, only high-quality genotypes (call rate > 95 %) were chosen. For each of the tested animals, the carrier status for the seven lethal Holstein haplotypes was determined by analyzing the existence of alleles that are included in the haplotype. The animal has been recorded as carrier for a haplotype if it has been identified the alleles combination for that haplotype.

Amplification of individual DNA fragments. All the samples were tested for HCD using the method described by (Menzi et al., 2016). The method can be summarized by using three pairs of primers: the wild type forward primer (WF) 5'-GGTGACCATCCTCTCTCTGC-3', the wild type reverse primer (WR) 5'-AGTGGAACCCAGCTCCATTA-3', and the mutant forward primer (MF) 5'-CACCTTCCGCTATTCGAGAG-3'. The primers WF and WR ensure amplification of the DNA fragment that does not contain the mutation (249 bp in size), while the WF and MF amplify the fragment that contains the insertion (436 bp in size).

PCR was performed under the following conditions: 3 min at 94 °C, followed by 35 cycles each consisting of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C, ending with 5 min at 72 °C. The PCR was performed for two reaction mixes, each of them being 10 µl in the final volume; the first mix contained 2 µl of 5 × Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 µl of HCD WF primer (2.5 pmole/µl), 0.4 µl of HCD WR primer (2.5 pmole/µl), and 6.2 µl of H_2O. The second mix contained: 2 µl of 5 × Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 µl of HCD MF primer (2.5 pmole/µl), 0.4 µl of HCD WR primer (2.5 pmole/µl), and 6.2 µl of H_2O.

Samples were tested for brachyspina mutation (HH0) using the allele-specific PCR method described by (Charlier et al., 2012). The first pair of primers, forward Across_UP1 5'-TCACAAAAGGGTAGGAGACTACCTG-3' and reverse Across_DN1 5'-GCTTATTGTTTACCCTTGACAGTGG-3', were used to amplify the DNA fragment

that does not contain the deletion. The size of the fragment is 551 bp. The second pair of primers ensures amplifying the fragment containing the mutation; forward BY Within_F1 5'-GCT-CAA-GTA-GTT-AGT-TGC-TCC-ACT-G-3' and reverse BY Within_R1 5'-ATA-AAT-AAA-TAA-AGC-AGG-ATG-CTG-AAA-3'. The fragment size is 421 bp (Charlier et al., 2012).

PCR for brachyspina was performed using the following conditions: 3 min at 94 °C, followed by 35 cycles each consisting of 30 s at 94 °C, 30 s at 58 °C and 30 s at 72 °C, ending with 5 min at 72 °C. The first PCR-mix (10 μl) contained 2 μl of 5 × Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 μl of Across_UP1 primer (2.5 pmole/μl), 0.4 μl of BY Across_DN1 primer (2.5 pmole/μl), and 6.2 μl of H_2O. The second mix (10 μl) contained 2 μl of 5 × Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 μl of BY Within_F1 primer (2.5 pmole/μl), 0.4 μl of BY Within_B1 primer (2.5 pmole/μl), and 6.2 μl of H_2O.

Three primer pairs were used to identify carriers for HH5 mutation. The (HH5_F) forward primer 5'-AGATATGCTAAAGTTTACCTAGAAGAA-3', and two reverse primers (HH5_WT_R) 5'-CTGAAGCTCCATTCTGAGTCAT-3', and (HH5_Del_R) 5'-TGCTCTATGAATTTTGTGAATGGT-3'. The primers HH5_F and HH5_WT_R were used to amplify the DNA fragment that does not contain the mutation producing a fragment, which is 442 bp in size, while HH5_F and HH5_Del_R amplify the fragment containing the mutation, and the size of the obtained fragment is 256 bp.

Two PCR reaction mixes were used, each of them being 10 μl. The first mix was 2 μl of 5× Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 μl of HH5_F primer (2.5 pmole/μl), 0.4 μl of HH5_WT_R primer (2.5 pmole/μl), and 6.2 μl of H_2O. The second mix contained 2 μl 5 × Mix (PCR-mix 5 × MasCFETaqMIX-2025), 0.4 μl of HH5_F primer (2.5 pmole/μl), 0.4 μl of HH5_Del_R primer (2.5 pmole/μl), and 6.2 μl of H_2O.

The PCR amplification products were analyzed on 4 % TAE-based agarose gel with a voltage of 120 V for 40 min using 1 × TAE (0.04 M Tris base, 0.02 M acetic acid, 0.5 M EDTA) buffer and ethidium bromide staining for visualization. The DNA Ladder M-50 (DIALAT Ltd., cat. no. MWM-50RL) was used for determining the size of fragments.

Results

Choosing the breed of animals for embryo production. The Black-and-White holsteinized breed was chosen for embryo production. Nowadays, there are more than 300 breeds of *Bos taurus* around the word (Durov et al., 2013); only 120 of them are breeds for milk production and only 30 breeds are the most widely spread across the world. The most common dairy breed in the world is the Holstein (Dunin et al., 2013). In the Russian Federation the most common dairy breeds are Black-and-White, Simmental, Kholmogory, Red-and-White, Holstein, Red Steppe, Ayrshire, and Yaroslavl. The animals of Black-and-White breed make up about 58 % of the total Russian dairy cattle population. The largest number of these animals is concentrated in the European part of Russia. The total number of dairy cattle in Russia is about 1.587 million; from them 939.5 thousands are Black-and-White animals.

According to the previous information and statistics, we can say that the Black-and-White breed is the best and most popular dairy breed in Russia, and it is necessary to start the genomic evaluation of breeding values for its animals. However, in order to improve the milk production in their herds, farmers usually use Holstein bulls for insemination. This has led to a high degree of holsteinization for this breed. In fact, the proportion of Holstein "blood" in the Black-and-White breed could be in excess of 50 % (Tikhonova et al., 2015).

Choosing bulls for embryo production. Assessment of semen quality of the bulls was based on the sperm concentration (the number of sperm in millions/mL), the mobility of sperm (in percent) taking into account the percent of progressive motility, non-progressive motility, and immobility sperm.

Embryo production. Five follicular aspirations were carried out for 36 donor cows. As a result, we obtained 379 cumulus-oocyte complexes (COCs); 322 of them were used for *in vitro* maturation (IVM). From the 322 COCs 100 embryos were obtained that reached the blastocyst stage (7th day of embryo development). Considering the quality of the embryos obtained, 80 embryos were selected and transferred to individual petri dishes for biopsy. From the 80 embryos, 78 biopsy specimens were obtained for which the whole genome amplification (WGA) was performed (Fig. 1).

Results of genotyping after WGA. The average concentration of DNA after amplification was 288.16 ng/μl (minimum 39.3 ng/μl, and maximum 567.4 ng/μl). Of the 78 samples, one had a concentration less than 50 ng/μl. The obtained DNA concentrations are comparable to those obtained by similar studies (Polisseni et al., 2010; Shojaei et al., 2014), and they are sufficient for carrying out genotyping by DNA microarray.

Genotyping was performed using BovineSNP50 V3 DNA Analysis BeadChip (Illumina, USA). All genotypes for the 78 samples were of satisfactory quality (call rate of sample > 95 %). 46 biopsy specimens of the 78 were identified as males and 33, as females. The distribution of DNA concentration values and the quality of genotyping of samples are shown in Fig. 2.

Identification of the carriers of lethal haplotypes. The genotypes for all the 78 embryo biopsy specimens were analyzed to identify the samples that are potential carriers of lethal haplotypes. Finding carriers for HH0 (brachypine), HH5 and HCD was expected since the frequencies of these haplotypes in the cattle population are higher than the frequencies of other haplotypes. The frequency of HH0 in the French Holstein cattle population is 7.4 % (Fritz et al., 2013), and it is 3.9 % and 6.7 % for HH5 and HCD, respectively, in the German Holstein cattle population (Schütz et al., 2016).

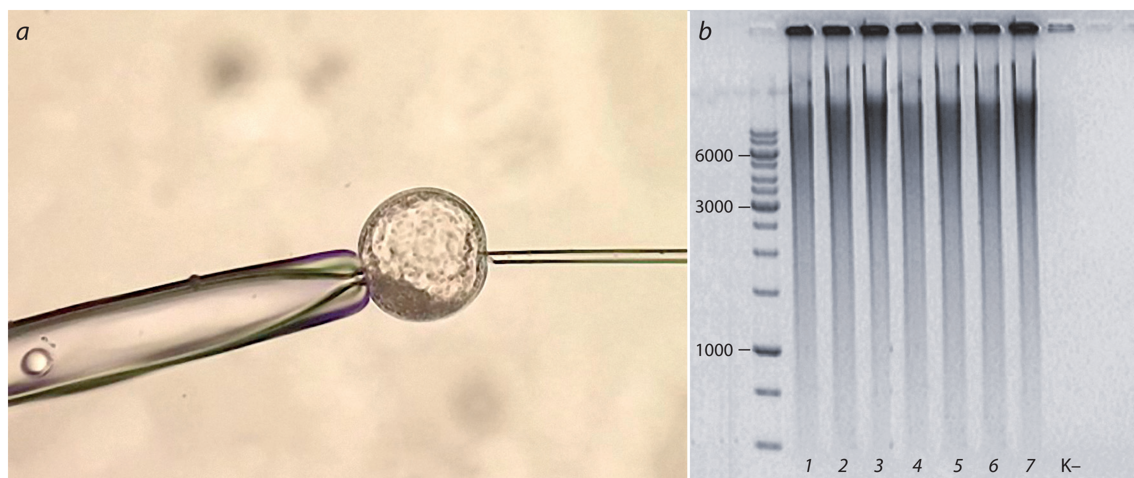

Fig. 1. Embryo biopsy and agarose gel electrophoresis for the WGA.

a – embryo biopsy using a needle; *b* – agarose gel electrophoresis for the products of the WGA (4 % gel, 120 V, 60 min, Thermo Scientific™ GeneRuler™ 1kb DNA Ladder 1kb DNA ladder, USA).

Among the 78 embryos, one carrier status for HH0, HH5, and HCD was detected in samples 14, 5, and 72, respectively.

The microarray probes do not contain DNA sequences for the direct detection of mutations associated with these lethal haplotypes, so the samples that were detected as being carriers of lethal haplotypes by analyzing the genotyping data were analyzed for the presence of casual mutations, using PCR analysis followed by gel electrophoresis. As a result, the casual mutation was confirmed by PCR analysis for the samples carriers for HH0 (brachispine) and HH5, while it has not been confirmed for the sample that is carrier for HCD (Fig. 3).

To confirm the status of carrier for HCD, but not carrier for the casual mutation, we tested the parents of this embryo for the presence of casual mutation for HCD. From the dam, we obtained hair for DNA extraction and sperms from the sire. As was expected, neither the mother nor the father were carriers for the HCD casual mutation (Fig. 4).

After genotyping and analyzing the genomic data for the presence of lethal haplotypes, the samples were tested for the presence of casual mutations using PCR (Fig. 5).

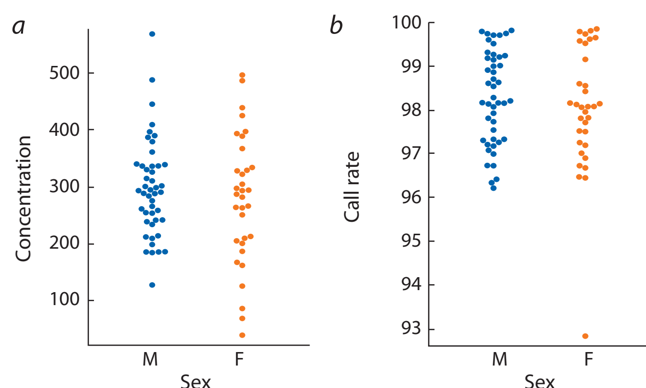

Fig. 2. The distribution of DNA concentration values and the quality of genotyping of samples (call rate).

a – DNA concentrations of biopsy specimens after the WGA; *b* – the quality of genotyping of biopsy specimens after the WGA.

Discussion

The situation in which an animal is carrier for HCD haplotype but not carrier of the casual mutation has been studied before and it is not associated with low quality of genotyping (the call rate of the genotype was 96.7 %). Large-scale studies on cattle populations have shown that animals that are homozygous for HCD can be either completely healthy or suffer from cholesterol synthesis deficiency. The pedigree analysis of these animals has shown that the primary source of the normal version of HCD haplotype is the bull HOCAN000000334489 WILLOWHOLME MARK ANTHONY born in 1975, and the casual mutation within this haplotype has occurred in its descendant, the bull HOCAN000005457798 MAUGHLIN STORM born in 1991. Thus, the bull HOCAN000005457798 MAUGHLIN STORM is considered the ancestor of the defective haplotype and its descendants carry the casual mutation, while the descendants of the bull HOCAN000000334489 WILLOWHOLME MARK ANTHONY whose family tree do not include the bull HOCAN000005457798 MAUGHLIN STORM are carriers for normal version of the haplotype, but not carriers for the casual mutation causing the disease (Kipp et al., 2015; Duff et al., 2016).

Conclusions

The results of this study show the feasibility to obtain a high quantity and quality of DNA after the whole genome amplification for embryo biopsy specimens. That indicates the possibility to perform high-quality genotyping on em-

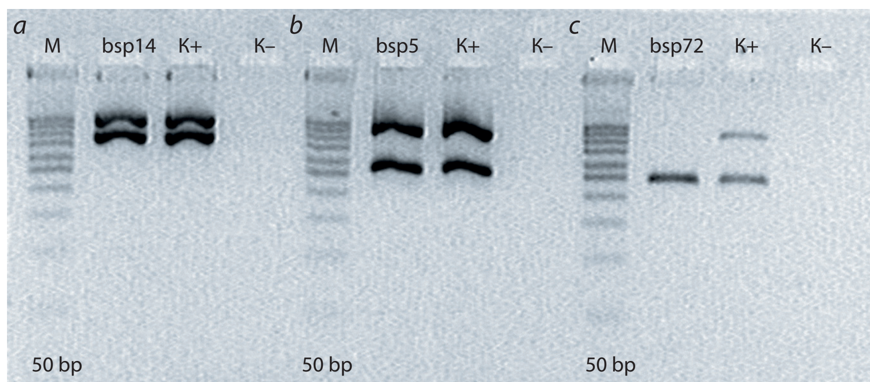

Fig. 3. Gel electrophoresis for PCR products for the samples carriers of lethal haplotypes according to microarray genotyping data.

a – HH0 (brachispine); *b* – HH5; *c* – HCD; M – 50 bp DNA marker; "K+" – positive control; "K–" – negative control; bsp14, bsp5 and bsp72 – samples analyzed.

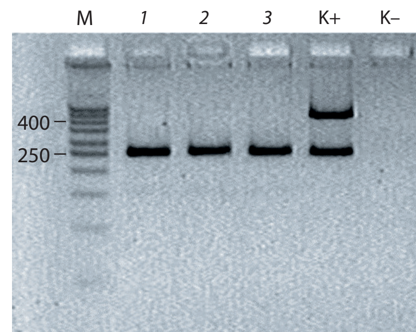

Fig. 4. Gel electrophoresis of PCR products for HCD.

1 – dam; *2* – sire; *3* – sample 72; M – 50 bp DNA marker; "K+" – positive control; "K–" – negative control.

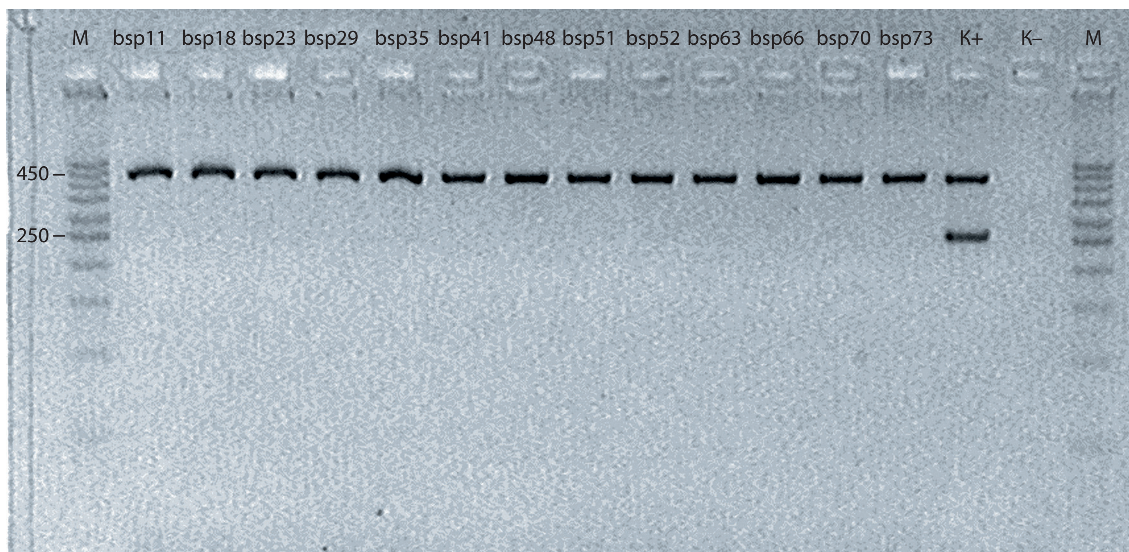

Fig. 5. Agarose gel electrophoresis of PCR products for HH5 casual mutation.

bsp11, bsp18, bsp23, bsp29, bsp35, bsp41, bsp48, bsp51, bsp52, bsp63, bsp66, bsp70, and bsp73 – samples analyzed. M – 50 bp DNA marker; "K+" – positive control; "K–" – negative control. According to the results, no carriers of HH5 casual mutation were identified.

bryos and to perform the genomic evaluation of animals at the embryo stage. The reliability of results obtained from the genotyping analysis can be confirmed by molecular genetic aspects using PCR methods – the classical methods for determining the carrier status of animals for monogenic diseases and lethal haplotypes. The embryos, from which genetic material was obtained and analyzed, were transferred to surrogate mothers. After the birth of animals, it is planned to verify the results obtained during the embryo analysis (gender, carrier status of monogenic diseases, and genotyping data) by re-genotyping the animals after birth. It is also planned to calculate the breeding value for these animals.

References

Boichard D., Ducrocq V., Croiseau P., Fritz S. Genomic selection in domestic animals: Principles, applications and perspectives. C. R. Biol. 2016;339(7-8):274-277. DOI 10.1016/j.crvi.2016.04.007.

Charlier C., Agerholm J.S., Coppieters W., Karlskov-Mortensen P., Li W., de Jong G., Fasquelle C., Karim L., Cirera S., Cambisano N., Ahariz N., Mullaart E., Georges M., Fredholm M. A deletion in the bovine FANCI gene compromises fertility by causing fetal death and brachyspina. PLoS One. 2012;7(8):e43085. DOI 10.1371/journal.pone.0043085.

Duff J.P., Passant S., Wessels M., Charlier C., Hateley G., Irvine R.M. Cholesterol deficiency causing calf illthrift and diarrhoea. Vet. Rec. 2016;178:424-425. DOI 10.1136/vr.i2265.

Dunin I.M., Adzhibekov K.K., Lozovaya G.S., Chekushkin A.M. Red-pied of dairy cattle in Russia. Farm Animals. 2013;1:56-61. (in Russian)

Durov A.S., Gamarnik N.G., Deeva V.S. Assessment of stud bulls in the population of the Simmental breed bred in the conditions of Khakassia. Vestnik Altayskogo Gosudarstvennogo Agrarnogo Universiteta = Bulletin of the Altai State Agricultural University. 2013;1:79-85. (in Russian)

Food and Agriculture Organization of the United Nations. Rome, 2007;4:381-427.

Fritz S., Capitan A., Djari A., Rodriguez S.C., Barbat A., Baur A., Grohs C., Weiss B., Boussaha M., Esquerré D., Klopp C., Rocha D., Boichard D. Detection of haplotypes associated with prenatal death in dairy cattle and identification of deleterious mutations in GART, SHBG and SLC37A2. PLoS One. 2013;8(6):e65550. DOI 10.1371/journal.pone.0065550.

Fritz S., Hoze C., Rebours E., Barbat A., Bizard M., Chamberlain A., Escouflaire C., Vander Jagt C., Boussaha M., Grohs C., Allais-Bonnet A., Philippe M., Vallée A., Amigues Y., Hayes B.J., Boichard D., Capitan A. An initiator codon mutation in SDE2 causes recessive embryonic lethality in Holstein cattle. J. Dairy Sci. 2018;101:1-12. Pubmed reference: 29680649. DOI 10.3168/jds.2017-14119.

Kipp S., Segelke D., Schierenbeck S., Reinhardt F., Reents R., Wurmser C., Pausch H., Fries R., Thaller G., Tetens J., Pott J., Piechotta M., Grünberg W. A new Holstein haplotype affecting calf survival. Interbull Bull. 2015;49:49-53.

Kuznetsov V.M. Historical Trends in Dairy Cattle Breeding in Russia and the USA. Kirov: North-East Research Institute of Agriculture Publ., 2015. (in Russian)

Ma L., Cole J.B., Da Y., VanRaden P.M. Symposium review: Genetics, genome-wide association study, and genetic improvement of dairy fertility traits. J. Dairy Sci. 2018 Sep. 26. pii: S0022-0302(18)30906- 8. DOI 10.3168/jds.2018-15269.

Menzi F., Besuchet-Schmutz N., Fragnière M., Hofstetter S., Jagannathan V., Mock T., Raemy A., Studer E., Mehinagic K., Regenscheit N., Meylan M., Schmitz-Hsu F., Drögemüller C. A transposable element insertion in APOB causes cholesterol deficiency in Holstein cattle. Anim. Genet. 2016;47(2):253-257. DOI 10.1111/age.12410.

Polisseni J., Sá W.F., Guerra Mde O., Machado M.A., Serapião R.V., Carvalho B.C., Camargo L.S., Peters V.M. Post-biopsy bovine embryo viability and whole genome amplification in preimplantation genetic diagnosis. Fertil. Steril. 2010;93(3):783-788. DOI 10.1016/j.fertnstert.2008.10.023.

Schütz E., Wehrhahn C., Wanjek M., Bortfeld R., Wemheuer W.E., Beck J., Brenig B. Correction: The Holstein Friesian lethal haplotype 5 (HH5) results from a complete deletion of TBF1M and cholesterol deficiency (CDH) from an ERV-(LTR) insertion into the coding region of APOB. PLoS One. 2016;11(6):e0157618. DOI 10.1371/journal.pone.0157618.

Shojaei Saadi H.A., Vigneault C., Sargolzaei M., Gagné D., Fournier É., de Montera B., Chesnais J., Blondin P., Robert C. Impact of whole-genome amplification on the reliability of pre-transfer cattle embryo breeding value estimates. BMC Genomics. 2014;15:889. DOI 10.1186/1471-2164-15-889.

Tikhonova T.N., Zharov I.N., Nikitina S.V., ..., Kharitonov S.N., Satsuk V.F., Kovalyuk N.V. Genetic Resources of ‘‘Moskovskoe’’ Enterprise for Breeding Work. Third Edition. Moscow, 2015;12. (in Russian)

VanRaden P.M., Olson K.M., Null D.J., Hutchison J.L. Harmful recessive effects on fertility detected by absence of homozygous haplotypes. J. Dairy Sci. 2011;94(12):6153-6161. DOI 10.3168/jds.2011-4624.

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