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The possibility of genetic evaluation of bovine embryos

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ABSTRACT

Improving the domestic dairy farming by increasing the average milk production from dairy cows is one of the main properties in the Russia Federation for these days. The use of modern technologies in animal breeding can accelerate the genetic improvement and increase the milk production from dairy cattle. In this study, it has been developed, for the first time in Russia, a system for genotyping the cattle embryos for the Russian Holstein breed (Black-and-white breed). Biopsy was performed for 200 embryos obtained from high-value donor cows and Holstein bulls. Biopsy specimens were genotyped using BovineSNP50 v3 DNA microarray. The average concentration of DNA after the whole-genome amplification (WGA) for the 200 biopsied samples was 277.01 ng/ µl. From the 200 biopsied embryos, 50 showed low-quality genotype (call rate < 85%). The average value of call rates for the other 150 embryos was 91.61%. From the 150 embryos, 60 were selected for transplanting. A total of 16 calves were born. The genotypes for calves highly matched (84.12% to 95.15%) those for corresponding embryos. The genomic estimated breeding values (GEBVs) were calculated for calves and corresponding embryos for 305-day milk yield trait using the single-step genomic BLUP (ssGBLUP) model.

The results showed a positive correlation between the genomic breeding values estimated based on genotypes of embryos and those obtained from genotypes of corresponding calves (r2 =0.93). The genomic estimated breeding values for calves highly matched those for the embryos especially when call rates for embryos were higher than 91%. These results indicate the possibility of estimation of the genomic breeding value for the Russian Holstein cattle at the embryo stage for the 305-day milk yield trait. That would reduce the costs of maintaining the young animals that are low-valued and accelerate the reproduction of high-value breeding animals.

Keywords: genotyping, cattle, genomic estimated breeding value, embryos, milk production, ssGBLUP, best linear unbiased prediction (BLUP), whole genome amplification.

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INTRODUCTION

In recent years, there is a need to increase the domestic milk production in the Russian Federation in order to cover the rapid growth of demand for dairy products (Kiely et al., 2015). Milk production can be increased by applying the breeding programs that are oriented to improve the milk production from dairy cows (Pantiukh et al., 2019). The use of modern technologies in animal breeding can accelerate the genetic improvement and increase the milk production from dairy cattle (Rexroad et al., 2019). The sequencing of the bovine genome in 2009 (Wiggans et al., 2009), and the availability of the genomic information to be used for calculating the animal breeding values have led to a revolution in the dairy cattle breeding (Weigel K, 2017). The first unofficial genomic evaluations were released in the United States in 2008 and became official for Holsteins, Jerseys, and Brown Swiss in 2009 (Wiggans et al., 2011). The implementation of genomic evaluation has led to an increase by 15–25% in the accuracy of estimation of breeding values, enabled to determine the genetic merit at a young age and to increase the selection intensity of animals. As a result, the genetic improvements have been accelerated in the animal populations and the efficiency of animal selection programs has been increased (Zinovieva et al., 2018).

The integration of genomic evaluation of breeding value with the modern reproductive technologies such as ovum-pick up and in vitro production (OPU-IVP) opens up even greater possibilities in animal breeding (Fisher, PJ, et al . 2012, Kadarmideen et al., 2015). Applying these technologies together enables to evaluate animals even at the embryo stage and to select only the embryos that are high in breeding values for transplanting (Fleming et al., 2018; Turner et al., 2019).

When performing genotyping for embryos, arises the problem of obtaining a sufficient amount of genomic DNA for genetic analysis. That is due to the reduced number of cells obtained from the biopsy. The number of cells that can be obtained during biopsy is extremely limited as it is necessary to preserve the viability of embryos (Polisseni et al., 2010). The approximate mass of DNA in one cell is about 6 pg, while Illumina BovineSNP50 v2 DNA analysis (Illumina, San Diego CA) requires about 300 ng of a good quality DNA for genetic analysis (Campos-Chillon et al., 2015). The technique that promises to overcome this problem is the whole genome amplification (WGA). By applying the WGA, it is possible to generate a sufficient quantity of genomic DNA in a sufficient quality to perform genotyping on DNA microarryes (Moghaddaszadeh-Ahrabi et al., 2012, Saadi et al., 2014). However, the whole genome amplification has also its limitations. In particular, many errors occur during amplification due to lack of identification of the alleles. This is known as allelic drop out, which often occurs as a result of a minimal amount of template DNA (Campos-Chillon et al., 2015).

The purpose of this study is to determine the possibility of performing genotyping of biopsy specimens for embryos of cattle and conducting a reliable genomic evaluation of the breeding value using the genomic information obtained from the embryos genotyping.

MATERIALS AND METHODS

In vitro production (IVP) of bovine embryos

Embryos production was performed using the method described by Pantiukh et al. (2019). The service bulls and donor cows were selected from the animals of the Russian Holstein breed. The bulls were selected based on the pedigree analysis, ancestors, breeding values and the quality of semen. One straw of 250 µl of semen was collected from each bull and transported in liquid nitrogen in a Dewar tank into the laboratory where the in vitro fertilization (IVF) was performed.

Donor cows were selected based on the age, production indicators of the cows and reproductive performance. Exhausted, obese and sick cows were excluded. An echographic characteristics analysis of the ovarian was performed to ensure obtaining large numbers of oocytes from each cow.

The chosen oocytes were those that met the following conditions: viable, evenly surrounded by cumulus cells, a fine-grained ooplasm that evenly fulfils the transparent shell of the oocyte, and

a homogenous thickness of the transparent shell with a round shape. Selected oocytes were set for maturation in media of in vitro maturation (IVM) for 22 h. After maturation, oocytes were washed from IVM media and transferred to fertilization medium. Spermatozoa were washed by centrifugation on a discontinuous 45:90 Percoll gradient and prepared for oocytes fertilization in vitro (IVF). Oocytes were kept for fertilization in the fertilization medium for 24 hours. Oocytes were cultivated on a palate incubator under a constant temperature, regulated humidity and gaseous environment. On the 6th day after cultivation, 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 cells were taken from the trophoblasts of the blastocyst. 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. Embryo viability was monitored for 8–24 h prior to cryopreservation.

Whole genome amplification (WGA)

The whole genome amplifiation was performed using isothermal multiple displacement amplification (IMDA) method and GenomiPhi V2 DNA amplification kit (Illumina, USA). The standard protocol was modified to obtain the optimal amount of DNA. A stage of cell lysis and a stage of purification of amplification products after WGA were added. Firstly, the following buffers were prepared: alkaline Lysis Buffer (100 µl: 5 µlof 1 M Dithiothreitol (DTT); 20 µl of 1M KOH; 75 µlof H2O), neutralizing Buffer (100 µl: 60 µl of 1 M Tris-HCl, pH 7.5; 40 µl of 1M HCl) and ethanol 70% (500 µl: 365 µl of ethanol 96%; 135 µl of H2o).

For cell lysis, 1.5 µl alkaline lysis buffer was added to each tube containing the biopsy cells and PBS buffer. Then, the tubes were incubated for 30 minutes at -70 °C, and then for 10 minutes at 65 °C. After that, a 1.5 µl of neutralizing buffer was added to each tube, and then the tubes were placed in an ice bath.

For amplification, 9 µl GenomiPhi buffer was added to each tube. The DNA was denatured at 95 °C for 3 minutes and then the tubes were cooled on ice. After that, for each sample was added a 10 µl of the reaction buffer containing deoxyribonucleotide triphosphate (dNTP), random hexamers, and Phi29 polymerase. The reaction mixture was incubated at 30 °C for 18 hours. After the amplification, Phi29 DNA polymerase was inactivated by heating at 65 °C for 10 minutes. After that, tubes with amplification products were stored at -20 °C until genotyping (Polisseni et al., 2010). After the WGA, the quantity and quality of DNA were measured using NanoDrop ND1000-Technologies-Inc, Wilmington, DE, USA for quantity control, and agarose gel electrophoresis for the DNA quality.

DNA genotyping and breeding value estimation

The DNA obtained after the whole genome amplification and the DNA extracted from calves after birth were genotyped using a BovineSNP50 v3 DNA Analysis BeadChip microarray (Illumina, USA), according to the manufacturer's instructions. The DNA for calves was extracted from the ear tissues according to the standard protocol QuickGene DNA tissue kit L (DT-L). The DNA concentration was adjusted to 50 ng/µl, and then 4 µl from each sample was taken for the genotyping.

The calculation of the genomic breeding value for the embryos and corresponding calves was performed for the trait of 305-day milk yield using the phenotypic and genotypic database of the Russian Holstein breed (Russian Black-and-White breed). The single-step genomic BLUP model (ssGBLUP) was used to calculate the genomic breeding value, which was carried out in Genoanalytics Company (Moscow).

Embryo transplantation

Embryos were transplanted to recipient cows considering recommendations provided by the Center for Experimental Embryology and Reproductive Biotechnology (Moscow, Russia) for receiving and transplanting cattle embryos (Popov et al., 2017). The OvSynch hormonal protocol was applied for recipient cows. This protocol enables to perform embryo transplanting at the precise optimum time without control of the ovaries and uterus (Nowicki et al., 2017). The intravaginal progesterone releasing devices PRID DELTA were inserted into the vagina of cows, and the cows were injected with an estrogen hormone. On the 8th day, the PRID DELTA devices were removed, and animals were injected with prostaglandin F2α (PGF2α). After two days, recipient cows were treated with gonadotropin-releasing hormone (GnRH) analog (Ovarelin) by administering an intramuscular injections. After 8 days, a rectal examination was performed to confirm the physical signs of estrus. Criteria of the effectiveness of the treatments was the presence of corpus luteum (CL) on one of the ovaries. An ultrasound examination was performed to ensure the absence of cysts in the corpus luteum. After the examination of corpus luteum, embryos were transplanted to the cows that had an active corpus luteum using the recto-cervical method. On the 30th day after the embryos transplantation, an ultrasound examination was performed to check the pregnancy using a rectal linear ultrasound probe.

RESULTS AND DISCUSSION

The whole genome amplification was performed for the 200 embryo biopsies. The average DNA concentration obtained after the WGA was 277.01 ng/µl. This amount of DNA is sufficient for genotyping on DNA microarrays (Illumina, 2015). Comparable results were obtained in the work of Pantiukh et al. (2019). A sufficient concentration of DNA after the whole genome amplification have also reported in other studies (Giardina et al., 2009; Han., 2012). Polisseni et al. (2010) reported the possibility of obtaining an average concentration of DNA up to 400 ng/µl after the WGA. DNA genotyping was performed using BovineSNP50 v3 DNA Analysis BeadChip microarray (Illumina, USA). The call rates values were served as a quality control of genotyping. The call rates for the embryo biopsies ranged from 61.68% to 95.50%. From the 200 embryo biopsies, 50 showed low quality of genotyping (call rate < 85%). The average value of call rates for the other 150 was 91.61%. The results of genotyping showed a positive correlation between the DNA concentrations obtained after the WGA and the values of call rate after genotyping. The coefficient correlation was 0.71 (Fig. 1).

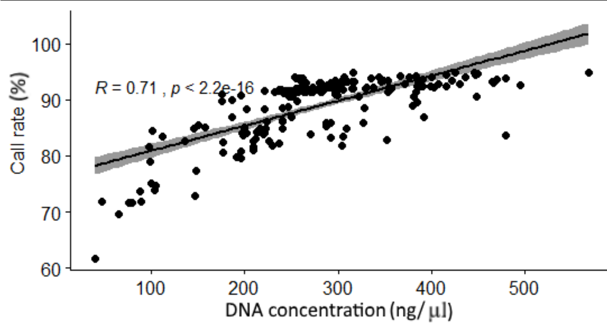

FIG. 1. THE RELATIONSHIP BETWEEN THE DNA CONCENTRATIONS AFTER THE WGA AND VALUES OF CALL RATE AFTER GENOTYPING ON DNA MICROARRAY.

For transplantation, 60 embryos with different values of call rate were selected. The call rates for the selected embryos ranged from 80.77% to 95.90%. On the 30th day after transplanting, an ultrasound scan was performed to check the pregnancy of recipient cows. Of the 60 recipient cows, pregnancy was detected for 33. That indicates to a pregnancy rate of 55%. The abortion was noticed for 17 cows at different periods of pregnancy. A total of 16 animals were born, 6 of them females and 10 males. The birth rate from the pregnant recipient cows was 48.5%. The results of embryos transplantation are presented in the table1.

TABLE 1. THE RESULTS OF EMBRYO TRANSPLANTATION.

Total number of biopsied embryos	Total number of transplanted embryos	Number of pregnant recipient cows on the day 30th	Pregnancy rate (%)	The number of born calves	Birth rate (%)
200	60	33	55	16	48,5

In the third month after birth, ear tissues were taken from calves. DNA from the ear tissues was isolated using QuickGene DNA tissue kit L (DT-L). DNA of calves was also genotyped on the BovineSNP50 BeadChip V3 DNA microarrays (Illumina, USA). The genotypes of calves were compared with those of the corresponding embryos. The call rate for all of the 16 calves was higher than 98%, while it ranged from 84.78% to 95.50% for the corresponding embryos. The results showed a high concordance rate (ranged from 84.12% to 95.15%) between genotypes of biopsied cells and genotypes of corresponding calves (table 2).

To assess the effect of difference between the genotypes of embryos and genotypes of corresponding calves, the single-step genomic BLUP (ssGBLUP) model was applied to calculate the genomic breeding value for the 305-day milk yield trait using the phenotypic and genotypic data of the Russian Holstein breed (Black-and-White breed). The single step genomic BLUP (ssGBLUP) has the potential to deliver more accurate and less biased genomic evaluations. It enables to include genotyped and nongenotyped animals simultaneously in the evaluation (Guarini et al., 2019). The reliability of estimation of genomic breeding value was calculated using the following formula (Thomasen et al., 2012):

$$REL = 1 - \frac{SE}{\sigma_a^2}$$

where σ_a^2 is the additive genetic variance and SE is prediction error.

The obtained results show a positive correlation between the genomic breeding values estimated based on genotypes of embryos and those obtained from genotypes of the corresponding calves. The coefficient correlation was 0.93 (Fig. 2). The results show that the genomic estimated breeding values for calves highly matched those for the embryos especially when the call rates for embryos were higher than 91%.

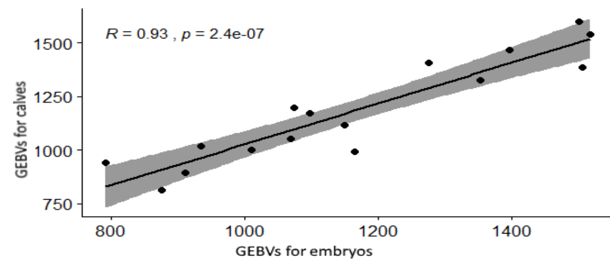

FIG. 2. THE RELATIONSHIP BETWEEN THE GENOMIC ESTIMATED BREEDING VALUES (GEBVS) FOR 305-DAY MILK YIELD (KG) FOR GENOTYPED EMBRYOS AND THOSE OBTAINED FROM GENOTYPES OF CORRESPONDING CALVES.

The results of estimations of genomic breeding value for calves and corresponding embryos and the accuracy of estimations are shown in the table 2.

TABLE 2. THE CALL RATES, CONCORDANCE RATES, GENOMIC ESTIMATED BREEDING VALUES (GEBVS) AND THE ACCURACY OF ESTIMATIONS OF GEBVS FOR GENOTYPED EMBRYOS AND CORRESPONDING CALVES FOR 305-DAY MILK YIELD (KG).

Sample	CR1 embryos (%)	CR 1 calves (%)	concordance rate 1	GEBVs embryos (kg)	Estimation accuracy GEBVs embryos	GEBVs calves (kg)	Estimation accuracy GEBVs calves
P6.12	84.78	99.32	84,12	1498.92	0.38	1599.68	039
P6.13	85.80	99.14	85,55	1395.8	0.39	1466.59	0.39
P6.18	85.99	99.22	85,89	1164.62	0.34	993.46	0.34
P6.19	86.24	99.29	85,95	1275.78	0.35	1408.25	0.35
P6.22	88.31	99.16	87,98	792.76	0.37	940.98	0.37
P7.16	88.37	99.19	88,28	1098.12	0.36	1172.23	0.38
P7.35	88.78	98.78	88,15	1074.71	0.39	1199.48	0.40

P7.38	89.81	98.82	88.40	1505.19	0.37	1387.43	0.37
P7.42	91.20	99.27	90.95	1352.23	0.35	1325.76	0.35
P7.47	91.32	99.15	91.10	1149.96	0.32	1115.85	0.31
P8.19	91.58	98.85	90.98	1516.41	0.35	1541.73	0.37
P8.21	91.94	98.84	91.66	1069.71	0.39	1053.28	0.40
P9.01	92.16	99,26	92.07	1010.65	0.33	1002.56	0.35
P9.11	92.98	99,23	92.50	911.65	0.34	895.33	0.36
P9.12	94.05	98,81	93.88	935.47	0.32	1016.98	0.32
P9.17	95.50	99,24	95.15	876.23	0.39	813.48	0.37

1 – Call rates in SNP genotyping. 2 – Concordance rates for called genotypes between biopsied embryos and corresponding calves

As shown in table 2, the difference between GEBV for biopsied embryos and GEBV for corresponding calves does not exceed 10% for the most of the samples, even when the levels of concordance rate are 85%. The difference between GEBV for biopsied embryos and GEBV for corresponding calves exceeded 10% only for two samples (P6.18 and P6.22). The accuracy of estimations of genomic breeding value was nearly the same for biopsied embryos and corresponding calves for all of the samples. The results could be interpreted by that, when using the ssGBLUP model, the effect of difference between the genotype of embryo and the genotype of corresponding calf could be minimized, since in the ssGBLUP model, not only the genomic information of the animal is included, but also, the pedigree information and phenotypic data (Guarini et al., 2019). The traditional inverse pedigree relationship matrix (A-1) in the mixed model equations in the traditional BLUP is replacing by the matrix H-1 in the ssGBLUP (Lee et al., 2019):

$$H^{-1} = A^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & G^{-1} - A_{22}^{-1} \end{bmatrix}$$

where A^{-1} - the inverse of the pedigree relationship matrix, A_{22}^{-1} - the inverse of the pedigree relationship matrix among genotyped animals, and G^{-1} - the inverse of the genomic relationship matrix.

The breeding value is the sum of a genomic and a polygenic genetic random effect, where the genomic random effects are correlated with a genomic relationship matrix G constructed from SNPs, and polygenic genetic random effects are correlated with the relationship matrix A. Thus, by using the pedigree data, it could be extended the genomic relationship matrix to non-genotyped animals or to genotyped animals that have some missed SNPs values (Christensen et al., 2010). In the ssGBLUP, the difference between the embryo and the corresponding calf occurs only in the G^{-1} matrix, while the A^{-1} and A_{22}^{-1} remain the same. Consequently, pedigree data in A^{-1} and A_{22}^{-1} matrixes could minimize the differences occurred in the G^{-1} matrix.

In a previous study, Mullaar et al. (2018) reported that the genomic estimated breeding values for the milk protein continent (kg) based on genotypes of the embryos is positively correlated with those values obtained by genotyping the corresponding calves after birth. The results show that the correlation is very high (r2 = 0.95) when only genotypes with a call rate above 0.85 are included. If embryos with lower call rates are included, the correlation is considerably lower (r2 = 0.71). In other study, FUJII et al. (2019) found that the GEBVs for carcass weight, ribeye area, and marbling score calculated from embryo biopsies closely matched those obtained from the corresponding calves. They reported the possibility of application of preimplantation genomic selection for carcass traits in Japanese Black cattle.

CONCLUSION

The genomic breeding value could be estimated at the embryo stage of cattle by conducting the biopsy of embryos and amplifying the DNA using the whole genome amplification method. A sufficient amount of DNA in a good quality for genotyping could be obtained after WGA. Genotypes of embryos and corresponding calves showed a high concordance rate from 84.12% to 95.15%. The differences in genotypes between biopsied cells and corresponding calves have not resulted in a large difference in the estimated breeding values. These results indicate the possibility of estimation of genomic breeding value for the Russian Holstein embryos for the 305-day milk yield. Based on the genomic estimated breeding values, embryos can been ranked, and only the highest valued embryos will be transferred. That would reduce the costs of maintaining the young animals that are low-valued and accelerate the reproduction of high-value breeding animals.

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