—Original Article—

Potential of preimplantation genomic selection for carcass traits in Japanese Black cattle

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Abstract. Preimplantation genomic selection using genomic estimated breeding values (GEBVs) based on single nucleotide polymorphism (SNP) genotypes is expected to accelerate genetic improvement in cattle. To develop a preimplantation genomic selection system for carcass traits in Japanese Black cattle, we investigated the accuracy of genomic evaluation of carcass traits using biopsied embryonic cells (Experiment 1); we also performed an empirical evaluation for embryo transfer (ET) of vitrified GEBV-evaluated blastocysts to assess the efficiency of the preimplantation genomic selection system (Experiment 2). In Experiment 1, the mean call rate for SNP genotyping using approximately 15 biopsied cells was $98.1 \pm 0.3\%$, whereas that for approximately 5 biopsied cells was $91.5 \pm 2.4\%$. The mean concordance rate for called genotypes between ~15-cell biopsies and the corresponding biopsied embryos was $99.9 \pm 0.02\%$. The GEBVs for carcass weight, ribeye area, and marbling score calculated from ~15-cell biopsies closely matched those from the corresponding calves produced by ET. In Experiment 2, a total of 208 *in vivo* blastocysts were biopsied (~15-cell) and the biopsied cells were processed for SNP genotyping, where 88.5% of the samples were found to be suitable for GEBV calculation. Large variations in GEBVs for carcass traits were observed among full-sib embryos and, among the embryos, some presented higher GEBVs for ribeye area and marbling score than their parents. The conception rate following ET of vitrified GEBV-evaluated blastocysts was 41.9% (13/31). These findings suggest the possible application of preimplantation genomic selection for carcass traits in Japanese Black cattle.
Key words: Carcass trait, Embryo, Genomic estimated breeding value (GEBV), Japanese Black cattle, Preimplantation genomic selection

Genomic selection in cattle based on high-throughput microarray platforms for genotyping tens of thousands to hundreds of thousands of single nucleotide polymorphisms (SNPs) is expected to accelerate genetic improvement, resulting in a shortened generation interval and increased reliability of predicted breeding values [1–3]. In dairy cattle, a genomic selection system has already been introduced for prediction of traits such as milk production, frame, and fertility in the selection of young bulls and heifers [4, 5]. Moreover, embryo transfer (ET) technology combined with superovulation as well as ovum-pick up with *in vitro* fertilization allows the accelerated propagation of genetic material from a selected dam, and these techniques combine well with genomic selection in cattle [6, 7]. In addition, preimplantation genetic diagnosis (PGD) enables the efficient production of progeny by increasing selection pressure and efficient use of recipient animals. To further improve the efficiency of cattle breeding, it is essential that a genomic selection system based on PGD is established.

An embryonic biopsy is a prerequisite for PGD and, since SNP genotyping takes at least several days, cryopreservation of the biopsied embryo is also necessary. In bovine embryos, an embryonic biopsy is generally performed at the blastocyst stage. As large biopsies lead to reduced viability and conception rates after cryopreservation and transfer [8, 9], the volume of cells that can be biopsied from a bovine blastocyst is limited to approximately 10% of the embryo (generally 10–15 cells). The quantity of DNA obtained from a biopsy of 10–15 cells is approximately 60–90 pg, which is insufficient for SNP genotyping based on microarray platforms. Therefore, whole-genome amplification (WGA) technology is necessary to amplify the input DNA prior to SNP genotyping. Several studies using dairy cattle have reported a high correlation between the genomic estimated

breeding value (GEBV) of economic traits (such as Lifetime Profit Index and kilograms of milk protein) in biopsied embryonic cells processed for WGA and the corresponding factors in calves produced by ET [10, 11]. These findings demonstrated the effectiveness of WGA prior to SNP genotyping for accurate GEBV calculation and the possible application for a preimplantation genomic selection system in dairy cattle.

Japanese Black cattle are the primary Wagyu breed, well known worldwide for its meat quality, especially the marbling score. Recently, a genomic evaluation system for carcass traits like carcass weight, ribeye area, and marbling score has begun to be established in Japanese Black cattle [12–14], and further acceleration of genetic improvement is expected in this breed using a preimplantation genomic selection system. To develop such a system for carcass traits in Japanese Black cattle, it is necessary to evaluate the accuracy of the GEBVs for carcass traits calculated from biopsied embryonic cells. It is also necessary to evaluate the efficiency of a preimplantation genomic selection system for carcass traits in Japanese Black cattle to ensure practicality of implementation.

In the present study, we investigated the accuracy of genomic evaluation for carcass traits in preimplantation embryos derived from Japanese Black cattle by comparing the SNP genotyping data and GEBVs for carcass traits in biopsied embryonic cells against the corresponding biopsied embryos or calves produced by ET (described as Experiment 1). In addition, we also investigated the variation in GEBVs for carcass traits among full-sib embryos and performed an empirical evaluation for ET of vitrified GEBV-evaluated *in vivo* blastocysts (described as Experiment 2).

Materials and Methods

Animal care

All experiments using animals were approved by the Animal Ethics Committee, Animal Research Center, Hokkaido Research Organization.

Production of bovine in vivo blastocysts

Donor cattle for *in vivo* blastocyst production (20 Japanese Black and 1 Angus cattle) were maintained at the Animal Research Center, Hokkaido Research Organization. Embryos derived from the Angus cow were used only in Experiment 1 to investigate the effect of input cell number on the accuracy of SNP genotyping. Controlled internal drug release devices (CIDR®1900, Pfizer Japan, Tokyo, Japan) were inserted into the vaginas of donors at any stage of the estrous cycle. After 2–4 days, 1 mg of estradiol benzoate (Eb, Kyoritsu Seiyaku, Tokyo, Japan) was administered to each cow. Starting 4 days after the Eb injection, follicle-stimulating hormone (FSH) (Antrin®-R10, Kyoritsu Seiyaku) was administered intramuscularly to each cow twice daily in decreasing doses over 3 days, to a total dose of 20 A.U. The CIDR was removed 48 h after the initiation of FSH treatment, and prostaglandin F2α (cloprostenol 0.5 mg/cow, Resipron®-C, ASKA Animal Health, Tokyo, Japan) was injected intramuscularly to induce luteolysis. Donor cows underwent artificial insemination (AI) at 12–24 h after estrus onset using frozen-thawed semen from seven Japanese Black sires. Blastocysts were non-surgically recovered by uterine flushing using balloon catheters (Fujihira Industries, Tokyo,

Japan) on days 7–8 (AI = Day 0). The recovered blastocysts were classified according to the International Embryo Technology Society (IETS) manual [15]. Only IETS Code 1 and 2 blastocysts were used for the experiments.

Blastocyst biopsies

Blastocyst biopsies were conducted in modified phosphate-buffered saline (PBS; Nissui Pharmaceutical, Tokyo, Japan) under an inverted microscope. The blastocysts were divided into approximately 15 biopsied cells and biopsied embryos or approximately 5 biopsied cells and biopsied embryos (Fig. 1A) using a micromanipulator equipped with a microblade (Feather Safety Razor, Osaka, Japan).

DNA extraction

DNA extraction from biopsied cells and embryos: Biopsied cells and biopsied embryos were washed three times in PBS supplemented with 0.02% polyvinyl alcohol (PVA; Sigma-Aldrich, St. Louis, MO, USA), and then once in PBS without PVA. The biopsied cells and embryos were collected individually into 0.2 ml tubes with 1.5 µl of PBS (Invitrogen, Carlsbad, CA, USA). DNA extraction was performed using the method built into the illustra™ Single Cell GenomiPhi™ DNA Amplification Kit (GE Healthcare, Tokyo, Japan) according to the manufacturer's instructions. Briefly, extraction buffer containing dithiothreitol (DTT) was added to lysed cells which were then incubated at 65°C for 10 min. The DNA extracted from biopsied cells was immediately processed for WGA, whereas that obtained from the biopsied embryos was stored at −80°C until SNP genotyping.

DNA extraction from blood and ear cells: DNA was extracted from calf or dam blood (dams A and B) and calf ear cells using the DNeasy Blood & Tissue Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The extracted DNA was quantified using a NanoDrop™ spectrometer (ND-1000; Thermo Fisher Scientific, Waltham, MA, USA) and stored at −30°C until SNP genotyping.

DNA extraction from sperm: Frozen-thawed sperm from two sires (sires A and B) were washed twice in 1 ml of washing buffer by centrifugation at 4°C, 5,800 × *g* for 5 min. The washed sperm pellets were resuspended in 800 µl of extraction solution containing 40 mM DTT, 0.08% SDS, and 0.5 mg/ml proteinase K and incubated at 50°C overnight. The samples were then incubated at 94°C for 15 min to inactivate the proteinase K and centrifuged at 4°C, 20,000 × *g* for 10 min. The supernatants were transferred to a clean tube, mixed with 500 µl of Tris-EDTA (TE) phenol (Wako Pure Chemical Industry, Osaka, Japan), and centrifuged at 4°C, 20,000 × *g* for 5 min. The supernatants were then mixed with 500 µl of chloroform (Wako) and centrifuged at 4°C, 20,000 × *g* for 5 min. Subsequently, the supernatants were mixed with 30 µl of 3 M sodium acetate (Thermo Fisher Scientific) and 750 µl of 99.5% ethanol (Wako), and centrifuged at 4°C, 20,000 × *g* for 20 min. The resulting sperm pellets were washed in 1 ml of 70% ethanol, rehydrated using vacuum oven for 40 min, and re-suspended in 200 µl of TE buffer (Invitrogen). The extracted DNA was quantified using the NanoDrop and stored at −30°C until SNP genotyping.

Whole-genome amplification

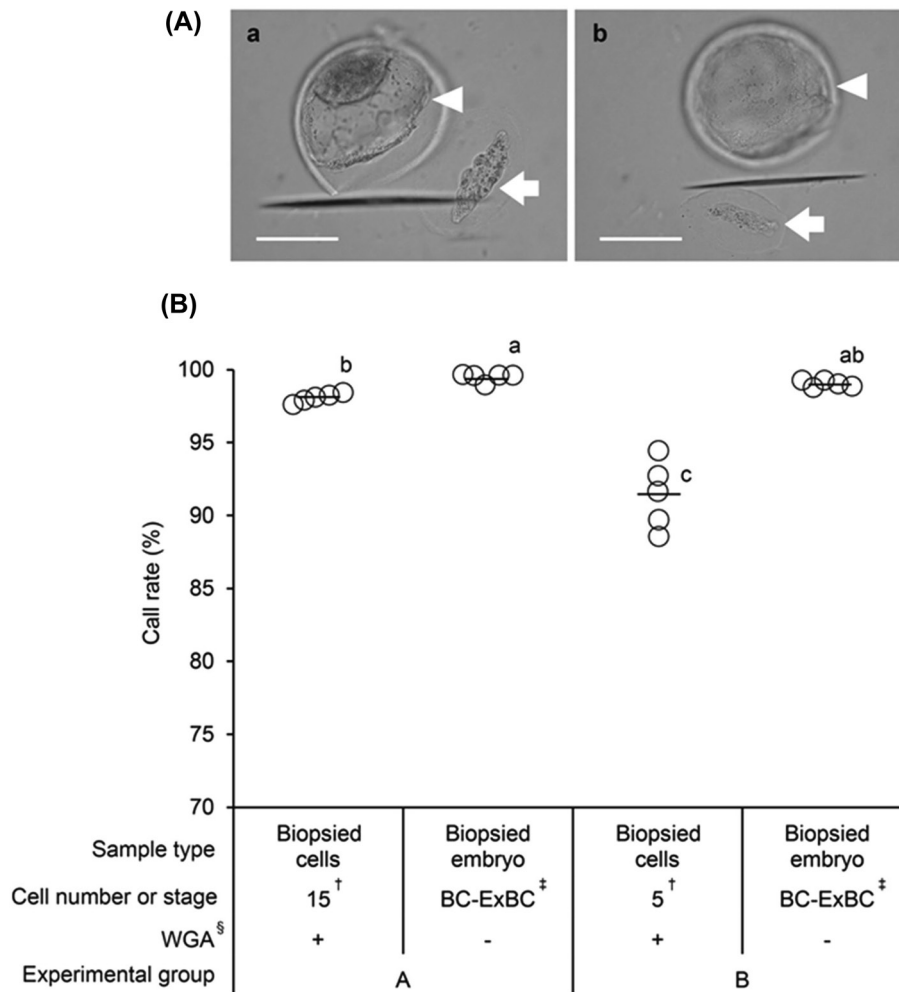WGA of DNA extracted from biopsied cells was performed using

Fig. 1. (A) Representative photographs showing a blastocyst biopsy (Day 7.5). The blastocyst was divided into biopsied cells (a: approximately 15 cells, b: approximately 5 cells) and biopsied embryos using a micromanipulator equipped with a microblade. Arrows indicate the biopsied cells. Arrowheads indicate the biopsied embryo. Scale bar = 100 μm. (B) Effect of input cell number on the call rates for SNP genotyping in biopsied cells. † Visually estimated approximate number. ‡ Blastocyst (BC) to expanded blastocyst (ExBC) biopsied. § WGA: Whole genome amplification. Lines indicate the mean values of call rates. Different superscripts indicate significant differences (P < 0.01).

the illustra™ Single Cell GenomiPhi™ DNA Amplification Kit (the operation of which is based on the multiple displacement amplification (MDA) method) according to the manufacturer's instructions. DNA processed for WGA (WGA-DNA) was quantified using the NanoDrop, diluted with TE buffer to a concentration of 50–70 ng/μl, and stored at −80°C until SNP genotyping.

SNP genotyping and calculation of GEBVs for carcass traits

To prepare the DNA for SNP genotyping, WGA-DNA derived from biopsied cells and DNA derived from biopsied embryos, blood, ear cells, and sperm were processed using the Infinium® HD Assay Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. SNP genotyping was performed using an Illumina bovine LD (7,931 SNPs) or 50K (53,113 SNPs, for sire B) BeadChip (Illumina). The SNP genotyping data was imputed, and a total of 34,481 SNPs were used to calculate GEBVs for carcass weight (kg),

ribeye area (cm^2), and marbling score. GEBVs were calculated by the genomic BLUP (GBLUP) method [16] using the phenotypic and genotypic data from 4,311 Japanese Black steers. Year of slaughter, meat market, and the linear and quadratic covariates of month of age were treated as fixed effects, and the SNP allele effects and residuals were treated as random effects in the GBLUP animal linear mixed model. Phenotype data were collected from 2003 to 2016 at three meat markets in Japan, namely Tokachi, Sibaura, and Nanko. Carcass traits were assessed according to the manual of the Japan Meat-Grading Association [17]. Sex determination was also performed using the SNP genotyping data.

Vitrification of biopsied embryos

Vitrification of biopsied embryos was performed according to the method described by Inaba *et al*. [18], with some modifications. Biopsied embryos were cultured in IVD101 medium (Research

Institute for Functional Peptides, Yamagata, Japan) in 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C for 2–3 h before vitrification. The embryos were then placed into an equilibration solution consisting of 7.5% ethylene glycol, 7.5% dimethyl sulfoxide (DMSO, Wako), and 20% newborn calf serum (NBCS) in TCM199 (Thermo Fisher Scientific) for 3 min, and then transferred into a vitrification solution consisting of 15% ethylene glycol, 15% DMSO, 0.5 M sucrose (Wako), and 20% NBCS in TCM199 for 40 sec. Biopsied embryos were subsequently placed on a Cryotop® device (Kitazato BioPharma, Shizuoka, Japan) with a small volume of vitrification solution (< 1 μl), immersed in liquid nitrogen, and preserved in liquid nitrogen until warming. Vitrified embryos were warmed in PBS (Invitrogen) supplemented with 0.2 M sucrose and 20% NBCS at 37°C for 3 min. Warmed biopsied embryos were cultured in IVD101 medium in 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C for 3–5 h to confirm their survival. Re-expanded and shrunken embryos were classified as viable and degenerated, respectively, after 3–5 h culture.

Embryo transfer

Recipient cows were estrus-synchronized by administration of a CIDR device for 7–14 days and an injection of prostaglandin F2α on the day of CIDR removal. Biopsied embryos were transferred to a uterine horn ipsilateral to the corpus luteum of recipient cows at 7–10 days after estrus using a YT gun (Yamanetech, Nagano, Japan). Pregnancy diagnosis was performed by ultrasonography at days 40 and 60 of gestation.

Experimental design

Experiment 1: Accuracy of SNP genotyping and GEBVs for carcass traits in preimplantation embryos

First, the effect of input cell number on the accuracy of SNP genotyping in biopsied cells was investigated using 10 blastocysts (IETS code 1). The blastocysts were divided into biopsies of either approximately 15 (experimental group A) or approximately 5 (experimental group B) cells, and biopsied embryos. The biopsied embryos were cultured in IVD101 medium in 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C until the next day. The DNA extracted from the biopsied cells was subjected to WGA and the WGA-DNA was then used for SNP genotyping. The DNA extracted from the biopsied embryos was processed for SNP genotyping without WGA. The accuracy of SNP genotyping in biopsied cells was determined by assessing the call rate and the concordance rate for called genotypes with the corresponding biopsied embryos.

Second, the concordances of GEBVs for carcass traits between biopsied cells and the corresponding calves produced by ET were investigated. Four blastocysts (IETS code 1) were divided into biopsies of approximately 15 cells and biopsied embryos. The DNA extracted from the biopsied cells was subjected to WGA. Biopsied embryos were freshly transferred to recipient cows, and DNA was obtained from the blood or ear tissue of delivered calves. WGA-DNA derived from the biopsied cells and DNA derived from the calves was processed for SNP genotyping and GEBV calculation. The concordances of GEBVs for carcass traits between biopsied cells and the corresponding calves were investigated.

Experiment 2: Evaluation of the efficiency of a preimplantation genomic selection system for carcass traits in Japanese Black cattle

A total of 208 *in vivo* blastocysts (IETS code 1 and 2) were biopsied (approximately 15 cells) and the biopsied embryos were vitrified. Biopsied cells were processed for WGA, SNP genotyping, and GEBV calculation. Only samples with call rates >85% were used for GEBV calculation.

First, GEBVs for carcass traits were compared among 12 full-sib embryos derived from two sire and dam couples (sire A × dam A and sire B × dam B).

Second, an empirical evaluation for ET of vitrified GEBV-evaluated blastocysts was performed, and the conception rate following ET was investigated.

Statistical analysis

In Experiment 1, the call rates and the concordance rate for called genotypes between biopsied cells and the corresponding biopsied embryos were subjected to arcsine transformation. Differences in call rates were analyzed by the Kruskal-Wallis test followed by multiple pairwise comparisons using Scheffe's method. The difference in the concordance rate for called genotypes between experimental groups A and B was analyzed using the Mann-Whitney U test. A probability value of P < 0.05 was considered statistically significant.

Results

Experiment 1: Accuracy of SNP genotyping and GEBVs for carcass traits in preimplantation embryos

The effect of input cell number on the accuracy of SNP genotyping of biopsied cells is shown in Fig. 1B. The call rates in biopsied embryos were >98.8%. In both experimental groups A and B, the mean call rates (mean ± SD) for the biopsied cells (experimental group A: 98.1 ± 0.3%, experimental group B: 91.5 ± 2.4%) were significantly (P < 0.01) lower than those for the corresponding biopsied embryos (experimental group A: 99.5 ± 0.3%, experimental group B: 99.0 ± 0.2%). The mean call rate for the ~15-cell biopsies was significantly (P < 0.01) higher than that for the ~5-cell biopsies. The mean concordance rate for called genotypes (mean ± SD) between the biopsied cells and the corresponding biopsied embryos in experimental group A (99.9 ± 0.02%) was significantly (P < 0.01) higher than for experimental group B (99.2 ± 0.4%).

As shown in Table 1, the call rates for the biopsied cells and the corresponding calves produced by ET were greater than 98.5% and 99.7%, respectively. The concordance rate for called genotypes between biopsied cells and the corresponding calves was 100% for all the couples. The relationship of the GEBVs for carcass traits between the biopsied cells and the corresponding calves is shown in Fig. 2. The GEBVs calculated from the biopsied cells closely matched those from the corresponding calves for all the carcass traits analyzed.

Experiment 2: Evaluation of the efficiency of a preimplantation genomic selection system for carcass traits in Japanese Black cattle

The SNP call rates for the biopsied cells ranged from 21.0% to 99.3%; the call rates in 88.5% (184/208) of the samples were > 85% (Fig. 3).

Considerable variation was observed in the GEBVs for carcass

Table 1. The call rates in SNP genotyping in biopsied cells and corresponding calves produced by embryo transfer and the concordance rate for called genotypes between biopsied cells and the corresponding calves

Embryo No.	Sample type [†]	WGA [‡]	Call rate [§] (%)	The concordance rate for called genotypes between biopsied cells and corresponding calf (%)
1	biopsied cells	+	98.6	100
	calf (blood)	−	99.7	
2	biopsied cells	+	99.3	100
	calf (ear cells)	−	99.7	
3	biopsied cells	+	99.2	100
	calf (blood)	−	99.8	
4	biopsied cells	+	98.5	100
	calf (blood)	−	99.7	

[†] Biopsy cells: approximately 15 cells, visually estimated. [‡] WGA: whole genome amplification. [§] Call rate was defined as the proportion of target SNPs giving positive signals on Illumina bovine LD. BeadChip analysis.
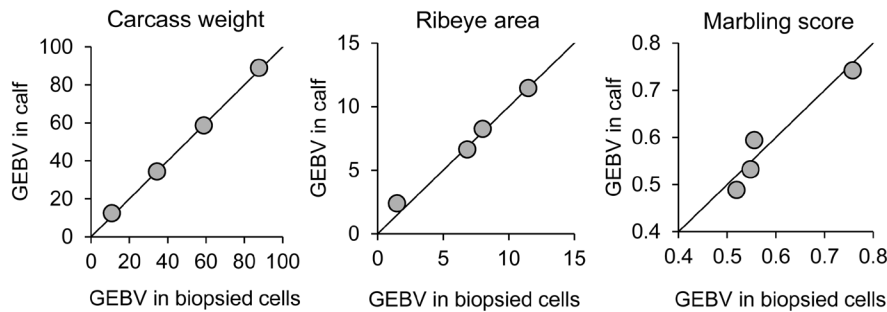

Fig. 2. The relationships of GEBVs for carcass weight, ribeye area, and marbling score between biopsied cells (approximately 15 cells) and the corresponding calves. Biopsied embryos were freshly transferred to recipient cows. The line in each graph indicates the formula: $y = x$.
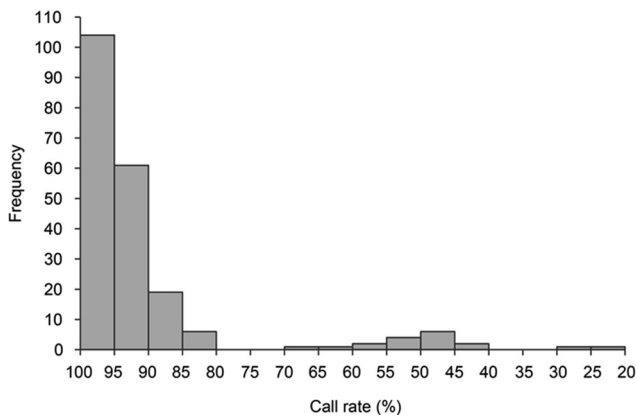

Fig. 3. Frequency of call rate in SNP genotyping in biopsied cells (a total of 208 embryos).

Based on GEBV records for carcass weight and marbling score, a total of 35 vitrified GEBV-evaluated blastocysts were selected and warmed. Of these, 31 embryos (88.6%) survived (re-expanded) after culture post warming (Table 2). Following ET, the conception rate at day 40 was 41.9% (13/31) (Table 2). No abortions were observed in any of the recipient cows until around day 60 of gestation (Table 2).

Discussion

Preimplantation genomic selection in cattle based on SNP genotypes is receiving increased attention as a means of accelerating genetic improvement by increasing selection pressure and avoiding the cost of producing genetically inferior animals [6, 19–21]. Although several groups have reported the potential of preimplantation genomic selection in dairy cattle [10, 11, 22–24], studies in Japanese Black cattle using this technique are limited. In the present study, we investigated the accuracy of GEBVs for carcass traits in preimplantation embryos derived from Japanese Black cattle. In addition, we investigated the GEBV variation in carcass traits among full-sib embryos and performed an empirical evaluation for ET of vitrified GEBV-evaluated blastocysts.

Although the PGD of limited loci, like those related to sex and genetic disorders, is possible using a small amount of DNA from a few biopsied cells [25, 26], SNP genotyping for genomic evalu-

traits among full-sib embryos derived from sire A × dam A (Fig. 4A) and sire B × dam B (Fig. 4B). For sire A × dam A, the GEBV for marbling score in 3 embryos was higher than that in sire A. For sire B × dam B, the GEBV for ribeye area in 4 embryos and marbling score, also in 4 embryos, was higher than in sire B.
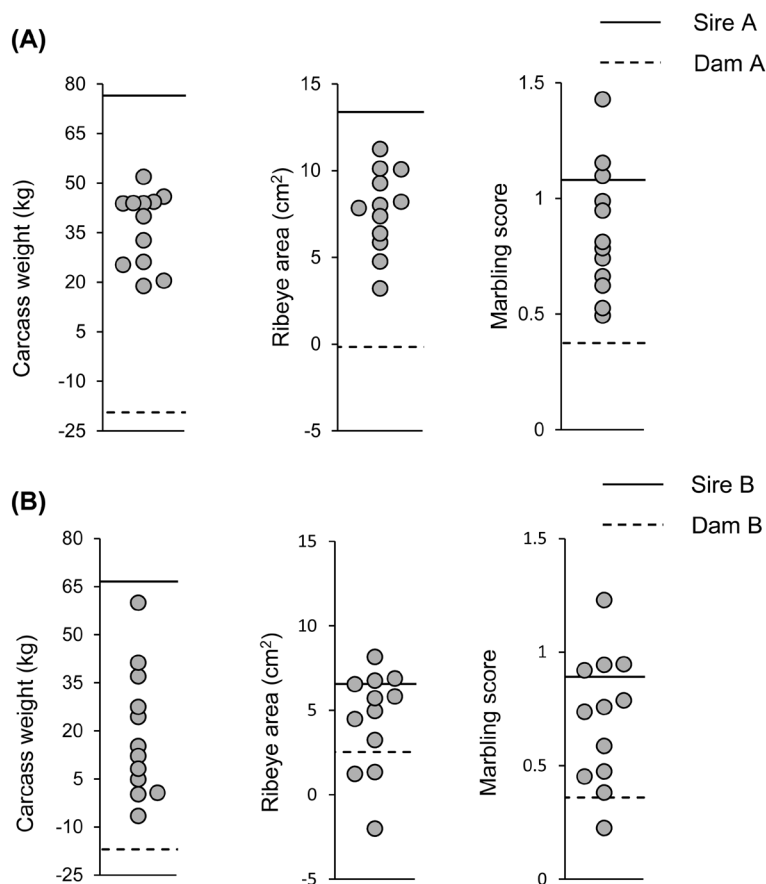
Fig. 4. Variation in GEBVs for carcass weight, ribeye area, and marbling score among full-sib embryos derived from Japanese Black cattle, namely sire A × dam A (A) and sire B × dam B (B). Only results in which call rates on SNP genotyping were > 85% are shown. The lines in each graph indicate the GEBVs for sire A or sire B. Dotted lines indicate the GEBVs of dam A or dam B.

Table 2. The conception rate following embryo transfer of vitrified GEBV-evaluated blastocysts

No. of GEBV-evaluated blastocysts warmed	No. of GEBV-evaluated blastocysts transferred [†] (%)	No. of cows pregnant (%)	
		Day 40	Day 60
35	31 (88.6)	13 (41.9)	13 (41.9)

[†] Re-expanded blastocyst after 3–5 h culture post warming.

ation, based on microarray platforms, requires a larger amount of DNA. Previously, Shojaei Saadi *et al*. [10] explored suitable WGA methodologies for SNP genotyping using biopsied embryonic cells (approximately 15 cells), and indicated that the illustra GenomiPhi V2 DNA amplification kit (GE Healthcare, MDA based method) produced the best results. Indeed, regarding the genetic value of the Lifetime Profit Index, their results showed an extremely high correlation (r = 0.99) between biopsied embryonic cells and the corresponding post-natal calves using the kit [10]. Correspondingly, we chose the illustra Single Cell GenomiPhi DNA Amplification Kit (GE Healthcare) for WGA in this study.

We used the blade biopsy method to obtain the biopsied embryonic cells in the present study. Prior to starting the study, we performed preliminary experiments to confirm the relation between biopsy volume and number of biopsied cells by nuclear staining test using Hoechst 33342. Thus, we assumed that our visual estimations of biopsied cell numbers were almost correct, although somewhat varied.

Illumina recommends that at least 200 ng of input DNA should be used for SNP genotyping. However, in this study, we observed a very high call rate (more than 98.8%) in biopsied embryos even without WGA, even though the amount of DNA derived was estimated at < 1 ng. Several studies [10, 11] observed strong inverse correlations between call rate and error rate in SNP genotyping; when the call rate is high, the genotyping error rate is low. In the present study, therefore, we considered that the results of the SNP genotyping of biopsied embryos without WGA were correct and could be used as a standard value against which to assess the accuracy of SNP genotyping in biopsied cells. In Experiment 1, using approximately 15 biopsied cells with WGA, the SNP genotyping call rates showed high values (more than 97.6%). In addition, the concordance rate for called genotypes between ~15-cell biopsies with WGA and the corresponding biopsied embryos were > 99.9%. These results indicate

that WGA prior to SNP chip analysis is effective for accurate SNP genotyping in biopsied embryonic cells as previously reported [10]. On the other hand, ~5-cell biopsies exhibited call rate and concordance rate for called genotypes between biopsied cells and the corresponding biopsied embryos significantly lower than for ~15-cell biopsies; nevertheless, WGA-DNA concentration in the ~5-cell biopsies was 710.9–746.1 ng/μl, levels similar to that observed in the ~15-cell biopsies (695.5–763.4 ng/μl), and was considered sufficient for SNP genotyping. Lauri *et al*. [9] reported that the missing calls and allele dropout rates increased when low amount of DNA template were used for WGA before SNP genotyping, an effect assumed to arise from an increase in amplification bias during WGA. Therefore, at least 15 biopsied cells should be used for SNP chip analysis to reliably obtain accurate SNP genotyping data.

In Experiment 1, we also demonstrated that GEBVs for carcass traits calculated from approximately 15 biopsied cells closely matched those for the corresponding calves produced by ET in all four couples. Ranks of GEBVs for all the carcass traits analyzed did not differ between biopsied cells and the corresponding calves. These results suggest that genomic evaluation for carcass traits using biopsied embryonic cells can be reliably performed in Japanese Black cattle.

Previously, Le Bourhis *et al*. [23] reported that when the call rate is > 84.7%, the concordance of genomic values for milk production and morphological traits between the embryo and the corresponding calf is very high. In addition, it has been reported that the correlation between the GEBV for kilograms of milk protein in biopsied cells and the corresponding calves was high ($r^2 = 0.95$) providing only samples with a call rate > 85% were used; if samples with a lower call rate were included, the correlation was considerably lower ($r^2 = 0.71$) [11]. These findings suggest that a call rate of > 85% may be the minimum threshold value for reliable estimation of breeding values in preimplantation embryos. In this study, therefore, we considered that a call rate of > 85% was suitable for GEBV calculation; samples with a call rate < 85% were omitted to avoid inaccurate GEBV calculation. In Experiment 2, the call rate was > 85% in 88.5% of the samples. In the remaining 11.5%, despite the concentration of WGA-DNA being sufficient for SNP genotyping (362.1–1136.3 ng/ μl), the call rate was < 85%. Although the reasons for a low call rate in false samples are not fully understood, possible reasons include that the number of biopsied cells was lower than estimated, that degenerated cells with fragmented DNA were included, and artificial operation errors. Careful handling of the samples and a high level of skill during embryo manipulation are required for reliable SNP genotyping and GEBV calculation. In addition, in Experiment 1, the call rates for approximately 5 biopsied cells were greater than 85%, from which the GEBVs could be calculated. However, setting the number of biopsied cells to approximately 5 would be expected to decrease the success rate of SNP genotyping. Consequently, we again suggest that at least 15 cells should be biopsied and used for SNP genotyping to maintain the efficiency of the preimplantation genomic selection system.

Interestingly, we demonstrated that there are large variations in GEBVs for carcass traits among full-sib embryos derived from Japanese Black cattle. Mullaart and Wells reported variation in GEBV for milk yield among full-sib embryos derived from dairy cattle [11]. Notably, we detected embryos presenting a higher GEBV for

ribeye area and marbling score than their sires. In the case of young individuals without progeny records, the expected breeding values are the average of the estimated breeding values of their parents. Thus, breeding values among full siblings are expected to be the same. Being able to distinguish the genetic ability among full-sib embryos at the preimplantation stage using GEBVs could have a marked impact on the efficient production of livestock with a high genetic ability for carcass traits.

In the present study, the conception rate following ET of GEBV-evaluated *in vivo* blastocysts was 41.9%. Inaba *et al*. [18] reported that conception rates measured between days 30–40 of gestation following ET of intact vitrified (using a cryotop device) *in vivo* bovine embryos was 46.7%. Studies conducted in collaboration with several Japanese prefectural livestock experimental stations previously demonstrated a pregnancy rate of 46.9% for biopsied (for sexing) and vitrified (using an ultra-rapid vitrification method) *in vivo* bovine embryos [27]. Although we did not perform a control experiment for ET (using intact vitrified embryos) and the conception rate obtained in this study using biopsied and vitrified embryos was only slightly lower than previously reported [27], we believe that the pregnancy rate we achieved would still be acceptable for application of a preimplantation genomic selection system. On the other hand, Lauri *et al*. [9] reported that the survival rate of bovine 30-cell biopsied embryos after cryopreservation (62%) was lower than that of 10-cell biopsied embryos (89%), indicating that the biopsied cells should be restricted to as few as possible [11]. Further studies are required to improve the accuracy of SNP genotyping based on using fewer biopsied cells for increasing and stabilizing the conception rate following ET of vitrified GEBV-evaluated blastocysts. Taken together with the success rate of SNP genotyping in biopsied cells, the variation in GEBVs for carcass traits among full-sib embryos, and the conception rate following ET of vitrified GEBV-evaluated blastocysts in Experiment 2 suggest that a preimplantation genomic selection system for carcass traits in Japanese Black cattle is feasible.

In conclusion, we have demonstrated a reliable genomic evaluation of carcass traits in Japanese Black cattle using biopsied embryonic cells and have shown that it is possible to select embryos with high genetic ability for carcass traits among full siblings using GEBVs. Moreover, we first showed the feasibility of using a preimplantation genomic selection system for carcass traits in Japanese Black cattle. Although further studies are needed to increase the accuracy of SNP genotyping using fewer biopsied cells and improve the production efficiency of GEBV-evaluated calves, the findings of the present study suggest a potential for the practical application of preimplantation genomic selection for carcass traits in Japanese Black cattle.

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