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GENOTYPING OF NELLORE BIOPSIED EMBRYOS

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SUMMARY

Genomic selection of embryos can boost genetic progress of beef cattle breeding programs by allowing the intensity of selection to increase and the generation interval to be reduced. This strategy depends on a protocol for the biopsy of embryos and DNA amplification, ensuring enough DNA for genotyping, without compromising the embryo's viability. In the present study, the quality of the genotypes of Nellore biopsied embryos was assessed based on genotyping call rate, Mendelian inconsistencies and allele dropouts. The results showed that the genotypes were of a good quality, suggesting feasibility of obtaining genomic prediction of Nellore embryos.

INTRODUCTION

Embryo transfer provides an outstanding opportunity for intensifying the production of genetically superior animals, given that donors and sires are properly selected. The technique of producing embryos of cattle through *in vitro* fertilization (IVF) has been evolving substantially and is becoming more reliable and accessible. For instance, according to the Brazilian Society of Embryo Technology, Brazil has been producing more than half a million embryos per year, through IVF.

Investments in IVF could be optimized if the genetic merit of the embryos could be predicted more accurately. Even if the donors and sires are properly selected, the embryo's genetic merit may substantially deviate from what is expected based on parent average, because Mendelian sampling is responsible for the genetic difference among full-sibs, and accounts for half of the additive genetic variability (Falconer and Mackay 1996). Genomic selection (Meuwissen *et al.* 2001) allows predicting more accurately the genetic merit of embryos, given that they are genotyped for a reasonable number of markers and that a good prediction equation is available. To genotype the embryos, a proper protocol to extract DNA must be developed, without compromising embryo's viability.

Pre-amplification of the DNA extracted from the embryos is required to provide enough DNA for the currently available genotyping platforms. The amplification process usually leads to reduced genome coverage which in turn results in some genotyping errors as, for example, allele dropout at heterozygous loci (Lauri *et al.* 2013). These errors could ultimately compromise the genomic prediction and the feasibility of performing genomic selection on embryos. An alternative to correct part of the genotyping errors is to also genotype the parents of the embryos and use this information to fix inconsistencies, followed by imputation to predict missing genotypes (Saadi *et al.* 2014).

In the present study, the feasibility of obtaining genomic prediction of Nellore biopsied embryos by evaluating the quality of their observed and imputed genotypes is assessed. Genomic predictions and their corresponding accuracies were also computed to envisage the potential benefit of applying genomic selection in Nellore embryos.

MATERIALS AND METHODS

Nellore embryos were produced from ovum pick-up from 28 donors and IVF using semen of two sires. These donors and sires are from a single beef cattle farm (Agropecuária Jacarezinho) which participates in the DeltaGen breeding program (www.deltagen.com.br). A total of 93 embryos were biopsied and genotyped. The biopsy of embryos and DNA extraction were performed according to a protocol developed by In Vitro Brasil S/A (www.invitrobrasil.com.br). The extracted DNA was amplified using commercial kits based on multiple displacement amplification (REPLI-g, Qiagen, Mississauga, ON, Canada). The Illumina Bovine 50K v2 chip (Illumina, San Diego, CA, USA) was used to genotype the embryos, donors and sires. The biopsied embryos were implanted into Nellore recipient cows and presented a pregnancy rate (31%) similar to the rate presented by a control group (24%), suggesting that the DNA extraction did not reduce the embryo's viability.

The software FImpute v2.2 (Sargolzaei *et al*. 2014) was used as in Saadi *et al*. (2014) to check for Mendelian inconsistencies, to fix some genotyping errors and to impute missing genotypes. As the parents of the embryos were also genotyped, family information was initially used by FImpute as the main source of information for fixing the inconsistencies. Afterwards, the fixed 50K genotypes were imputed to HD genotypes (Illumina Bovine HD chip), using family and population information. Finally, the embryos had their direct genomic values (DGV) calculated based on their imputed genotypes and on the prediction equation of DeltaGen breeding program. The reference population for imputation and genomic prediction used in this study has approximately eight thousand animals. The DGVs and their accuracies were calculated using the software GEBV (Sargolzaei *et al*. 2013). The analyses were performed using 34,900 SNPs from the 50K chip and 615,397 SNPs from the HD chip, comprising those SNPs which passed quality control of routine genomic evaluation.

The quality of the genotypes of embryos was mainly assessed by the comparison between observed and imputed genotypes. A better assessment will be performed after the resultant calves are born and genotyped, so the comparison will be made among the embryo-calf pairs.

RESULTS AND DISCUSSION

The average call rate of the embryos' 50K genotypes was equal to 0.93, ranging from 0.75 to 0.98. Seventy four embryos (80%) seemed to present a reasonably good quality of their genotypes (call rate≥0.90). Embryos' genotypes with lower call rates also presented lower levels of heterozygosity (Figure 1), suggesting low quality of the inferred genotypes and the occurrence of allele dropouts at heterozygous loci. Seventeen embryos, most of which with low call rate, showed parentage conflicts based on the original 50K genotype, showing more than 319 Mendelian inconsistencies when their genotypes were contrasted with those from their parents. The poor genotype quality (call rate<0.90) of some embryos were likely caused by the low amount of extracted DNA and the amplification process.

All the parentage conflicts were no longer observed using the imputed ("fixed") genotypes. The FImpute software corrected the Mendelian inconsistencies and imputed all missing genotypes, i.e. the call rate of the imputed genotypes was equal to 1, for both chips (50K and HD). Considering just the SNPs from the 50K chip (for a proper comparison) the level of heterozygosity of the imputed genotypes was, generally, greater than those of the observed genotypes, especially for the embryos originally presenting low call rates. This result indicates that FImpute was able to correct at least part of the allele dropouts. However, even after imputation, there was evidence of underestimation of heterozygosity for the embryos with original genotypes exhibiting low call rates (Figure 1).

Besides fixing the allele dropouts, FImpute uses family and population information to also correct some homozygote SNPs which were miscalled as heterozygotes or as the opposite

homozygote. Figure 2 shows that embryos with lower original call rates presented a higher percentage of genotypes corrected due to dropouts and total error rate. In general, the dropouts were responsible for the greatest proportion of changes. The maximum percentage per sample of homozygote SNPs miscalled as heterozygotes or as the opposite homozygote was equal to 0.4% and 0.5%, respectively, whereas it was equal to 1.9% for the dropout. The maximum total change per sample was equal to 2.6% (Figure 2).

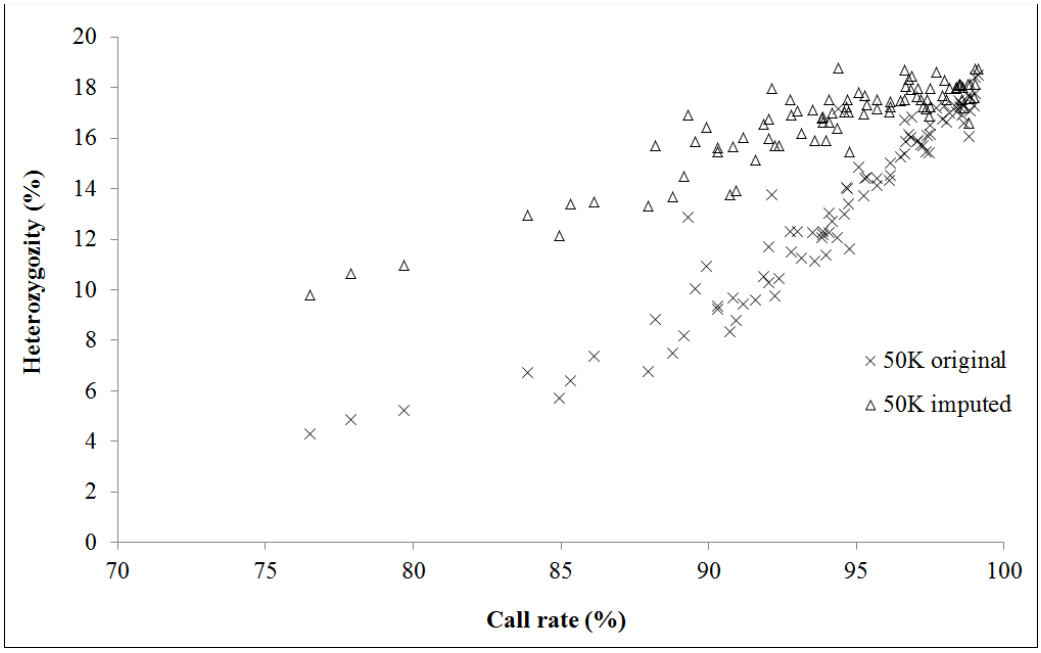

Figure 1. Call rate of original genotypes (%) and heterozygosity (%) of original and imputed 50K genotypes.

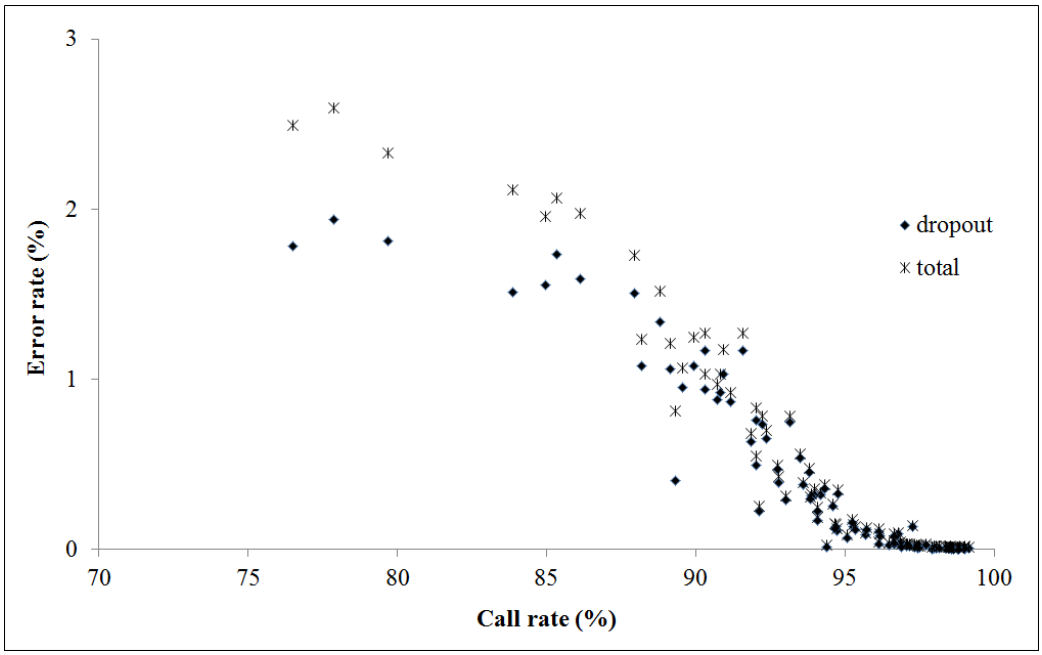

Figure 2. Call rate (%) and error rate (%) of embryos' genotypes.

It is important to emphasize that having good DNA extraction and amplification protocols remains very important. Even if imputation may improve genotype quality, it can also cause some errors and bias the genomic predictions. Pimentel *et al.* (2015) provided empirical evidence that top animals may have their genomic predictions underestimated when imputed genotypes are used, mainly due to miscalling low frequent haplotypes that could not be determined unambiguously by the imputation algorithm. As mentioned previously, the quality of the genotypes of embryos will be better assessed after the resultant calves are born and genotyped, so the comparison will be made among the embryo-calf pairs.

The genomic prediction of the embryos obtained after fixing and imputing their genotypes presented an average accuracy of 0.56 (ranging from 0.46 to 0.60), for the selection index used by the breeding program. This accuracy is equivalent to those for young bulls selected (without genomic information) for progeny testing, highlighting the potential benefit of applying genomic selection in Nellore embryos. The cost-effectiveness of this strategy is highly determined by the realized success rate of the transferred biopsied embryos.

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