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BRIEF COMMUNICATION: Potential for genomic selection of bovine embryos

PJ Fisher[a], DL Hyndman[a], MJ Bixley[a], FC Oback[b], L Popovic[b], LT McGowan[b], MC Berg[b] and DN Wells[b]*

[a]*AgResearch Invermay, Private Bag 50-034, Mosgiel 9053, New Zealand;* [b]*AgResearch Ruakura, Private Bag 3123, Hamilton 3240, New Zealand*
Corresponding author. Email: david.wells@agresearch.co.nz

Keywords: embryo; bovine; IVF; biopsy; vitrification; genomic selection; SNP; genotyping

Introduction

Sexual reproduction is a genetic lottery. There is no guarantee that offspring receive a favourable combination of genes from their parents to form their genotype. For animal breeding, the conventional way of selection has been based on phenotyping adults or their progeny. Increasingly, however, dairy cattle breeding schemes are integrating the use of genomic selection of juvenile and adult candidates (Hayes et al. 2009; Pryce et al. 2010). To further increase the intensity of selection and accelerate the rate of genetic gain, we aim to genomically select bovine embryos within one week after *in vitro* fertilisation (IVF) using gametes from elite parents. The generation of multiple embryos from multi-factorial *in vitro* matings allows for the selection of those possessing the best genotypes before transfer to recipient females and the generation of animals for future breeding (Georges & Massey 1991; Humblot et al. 2010).

Here, we report our initial investigations genotyping embryo biopsy samples, with low amounts of starting DNA template, on low and medium density bovine single nucleotide polymorphism (SNP) chips. This is complemented by studies determining the *in vivo* viability of biopsied bovine embryos that have been temporarily cryo-preserved, which is currently required to manage the logistics of embryo genomic selection in practice.

Materials and methods

Bovine IVF embryos were produced according to standard methods (Misica-Turner et al. 2007) using either Y-sorted sperm in Experiment 1, or non-sorted sperm in Experiments 2 and 3.

Whole genome amplification from previously frozen (-80ºC) embryo cell samples was performed using the GenomiPhi V2 DNA amplification kit (GE Healthcare, Piscataway, NJ, USA) according to manufacturers' recommendations, but without denaturation. Amplification success was confirmed both by quantification and visualisation of an aliquot of the DNA product on agarose gels. The standard Infinium II (Illumina, San Diego, CA, USA) SNP chip genotyping procedure was performed, including an initial 20h linear pre-amplification step. Genotypes were scanned using an iScan scanner and visualised using the accompanying GenomeStudio software (Illumina, San Diego, CA, USA). Standard settings were typically used. However, in-house scripts were also produced to aid separate clustering on intensity and green-red fluorescence ratios. The call rate, representing the proportion of SNPs that produce a viable genotype, and the replication error rate between the biopsy and the control embryo DNA, were the two metrics selected to determine genotype quality. In Experiment 1, the bovine 50K SNP chip was used, whereas in Experiment 2 the bovine 7K low density SNP chip was tested.

Experiment 1: Genotyping one- and three-cell biopsies from bovine morulae

Six transferable quality compacted morula-stage embryos were selected five days after IVF. Each embryo was disassociated and one- and three-cell samples for whole genome amplification and SNP genotyping provided in triplicate. The remainder of the embryo, which ranged between 12–40 cells and represented at least half the embryo mass, provided the control DNA for each set of biopsies.

Experiment 2: Genotyping biopsies from bovine blastocysts

The trophectoderm was biopsied from fifteen transferable quality blastocyst-stage embryos using a microsurgical knife (Bioniche, Belleville, Ontario, Canada) eight days after IVF. Following biopsy, each blastocyst was then bisected; thus generating three samples per original embryo. Precautions were taken to avoid cellular contamination between embryos. It had been previously determined from 44 separate embryos that such biopsies contained an average of 15 ± 1.1 cells and we estimated each bisection to contain 50 cells (Misica-Turner et al. 2007).

Experiment 3: Blastocyst biopsy and vitrification

IVF blastocysts were firstly biopsied as described above and then cryo-preserved using the Cryologic vitrification method (Fry et al. 2005). Vitrification involves the ultra-rapid cooling of embryos in a concentrated cryo-protectant solution. Following storage in liquid nitrogen, embryos were warmed to 38.5ºC and incubated for at least two hours to evaluate re-expansion of the blastocoel cavity and assess morphology before embryo transfer. Two embryos from either the biopsied and vitrified, or fresh control embryo treatment groups were transferred to the uterine horn ipsilateral to the *corpus luteum* of each synchronised recipient heifer. Fetal heartbeats were detected by ultrasonography to determine *in vivo* developmental potential up to

Table 1 Average call rate ± standard deviation and replication error ± standard deviation, in embryo biopsies in Experiments 1 and 2. P values in bold indicate significance between sample types. n/a = Not applicable.

Experiment	Sample	Number of biopsies	Call rate (%)	Replication error (%)
Experiment 1	1-cell biopsy	17	77.6 ± 6.2	7.8 ± 3.5
	3-cell biopsy	18	85.5 ± 2.8	2.9 ± 1.7
	> ½ morula (12–40 cells)	6	96.3 ± 1.7	n/a
	Standard error of difference		<2.0	0.8
	P value		**<0.001**	**<0.001**
Experiment 2	Trophectoderm biopsy	15	88.3 ± 5.0	1.1 ± 1.5
	Bisected blastocysts[1]	29	93.9 ± 3.7	0.1 ± 0.1
	Standard error of difference		1.2	0.4
	P value		**<0.001**	**<0.05**

[1]One bisected blastocyst lost before whole genome amplification.

Table 2 Number and pecentage of transferred embryos surviving on Days 35, 45 and 65 of gestation following blastocyst biopsy and vitrification in Experiment 3.

Treatment	Number of embryos transferred	Number of embryos surviving		
		Day 35	Day 45	Day 65
Biopsied and vitrified blastocysts	42	26 (62%)	24 (57%)	18 (43%)
Fresh control blastocysts	42	23 (55%)	19 (45%)	16 (38%)

Day 65 of gestation; after which, pregnancies were terminated. All animal manipulations were approved by the Ruakura Animal Ethics Committee.

Statistical analyses

Genotypic data were analysed using linear models and embryo survival data with Fisher's exact test.

Results and discussion

Following GenomiPhi amplification, the quantity and the size of the DNA products were generally considered acceptable, although single-cell biospies sometimes resulted in lower amounts of DNA. This did not necessarily correspond to a poor genotype result; however, one of the 18 single-cell samples (in Experiment 1) failed to yield a genotype and was not included in subsequent analyses.

With greater numbers of embryonic cells provided in the sample, the average call rate increased significantly while the average replication error decreased significantly (Table 1) similar to previous reports (Humblot et al. 2010). The call rate for 30–40 cell embryo samples in Experiment 1 approached the 99% rates typically achieved for parental DNA obtained from blood. For both the 7K and 50K SNP chips, there was a strong inverse relationship between call rate and replication error ($r^2 > 0.95$) indicating that a high call rate, which does not require replicates or references to compare against, is a good proxy for a low replication error. The genotypic error rates from one- and three-cell biopsies may be biased upwards because the replication error compares the test samples to the respective multi-cell embryos and while these reference samples have higher call rate and fewer errors, they are not error-free. There was no obvious effect from the stage or morphological quality of the embryos, or corresponding biopsies, on the call rate and replication error parameters.

As observed elsewhere (Humblot et al. 2010), we noted that most of the errors were due to allelic dropout, where a heterozygous embryo genotype actually resulted in a homozygote call in the biopsy. However, some errors appeared to be the result of the opposite phenomenon, where the homozygote was called incorrectly as a heterozygote in the biopsy, termed a false heterozygote. These false heterozygotes are likely to be more problematic than allelic dropouts, if imputation from parental SNPs is used to correct for missing genotypes. From the 50K SNP data in Experiment 1, three sets of three-cell biopsies were compared to their respective embryos, those deemed to have the fewest errors, to determine whether the replication error was due to allelic dropout or a false heterozygote. After adjusting for potential allelic dropouts in the reference embryo samples, from a total of 491,481 genotype pairs, 24,020 were determined to be allelic dropouts and another 1,740 classified as false heterozygotes

amongst these nine embryo biopsies. That is, false heterozygotes comprised 6.8% of the replication errors. A similar analysis was performed with the 7K SNP data from Experiment 2. From 103,635 genotype pairs, 1,988 replication errors were observed between the 15 bisected embryos and their respective biopsies. Once again, the majority of the replication errors were caused by allelic dropout. After adjusting for probable allelic dropouts in the bisected embryos, just 54 SNPs were likely to be false heterozygotes; thus averaging less than 4 SNPs per biopsy, or 2.8% of the replication errors.

In the embryo transfer trial, 96% (54/56) of blastocysts were successfully biopsied and deemed suitable for vitrification. Following warming, 50 (93%) biopsied vitrified embryos were recovered from liquid nitrogen storage. After a brief period of *in vitro* culture, 88% of embryos (44/50) were morphologically assessed to be of a quality suitable for transfer to recipients. Current research is directed towards further improving the cryo-tolerance and recovery rate of vitrified embryos. Importantly, the rate of *in vivo* development for those biopsied and vitrified blastocysts that were transferred was not significantly different compared to fresh control embryos, up to Day 65 of gestation at least (Table 2; 18/42 = 43% *v.* 16/42 = 38%, respectively). This compares favourably to rates reported elsewhere (Fry et al. 2005).

It appears that blastocyst biopsies provide sufficient cells to enable reasonably robust whole genome amplification and SNP genotyping. The 7K bovine low density SNP chip appears to be a useful tool for embryo genotyping, with an average error rate of ~1% per biopsy. Addition of missing genotypes and correction of errors in the biopsies should be manageable through the use of imputation software based on known, more densely genotyped parental SNPs. Future work will investigate genomic selection from embryo biopsy samples derived from oocytes collected from cows following trans-vaginal ovum pick-up, rather than the abattoir-derived material used in this study. The SNP genotypes of resulting calves will be compared to those from the original embryo biopsies to determine the reliability of this embryo genomic selection approach. Further optimisation of *in vitro* embryo culture systems, and especially improved fertilisation and development rates with sex-sorted sperm, will maximise the number of embryo genotypes produced and thus increase the selection intensity possible.

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