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INTEGRATION OF IVF TECHNOLOGIES WITH GENOMIC SELECTION TO GENERATE HIGH MERIT AI BULLS: A SIMULATION STUDY

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SUMMARY

A stochastic simulation model was used to determine the impact of incorporating genomic selection into the IVF process to generate high merit young bull candidates, from a breeding company perspective. IVF candidates were simulated with varying selection proportions applied at the blastocyst stage, then combined with standard genomic selection scheme candidates with the top 50 bulls selected on EBV using truncation selection. Selecting a low proportion of genotyped blastocysts for cloning was key to producing high EBV young bull candidates where the cost of the IVF technologies would be offset by a large reduction in rearing costs through having fewer candidates to rear through until semen producing age.

INTRODUCTION

Dairy cattle breeding is a highly competitive business driven by commercial semen companies that have generated substantial genetic gains. Genomic selection has been widely used in dairy cattle improvement systems since 2008, with recent estimates of the reduction in generation interval ranging from 7 months for dams of cows up to 4.5 years for the sires' of bulls' pathway (Garcia-Ruiz et. al., 2016), accompanied by substantial improvements in selection pressure on key traits. These improvements are based on genomic testing of young bull candidates to predict performances earlier along with improvements in the accuracy of genomic breeding values, particularly in lower heritability traits.

Fisher et al (2012) proposed a method of genotyping bovine embryo biopsies, with rates of *in vivo* development not significantly different to fresh control embryos. Carrying out genomic selection at the blastocyst level would allow for intense selection for favourable genotypes within a set of candidate embryos. This could be exploited within a commercial breeding program, either through increased selection intensity at an earlier stage, or through reduced rearing costs to identify top young AI bull candidates because only blastocysts of sufficient predicted genetic merit would be reared through to semen production and beyond.

This study used simulation to determine the impact of incorporating genomic selection into the IVF process. A breeding company perspective was taken, whereby elite young bull candidates were generated within a genomic selection breeding scheme.

MATERIALS AND METHODS

A stochastic simulation framework was developed using the python programming language, and with a generic parameterisation based on industry statistics of ages of young bulls. The simulation framework starts by using a burn-in phase to model a base population selection pool for a genomic selection breeding scheme, followed by various scenarios incorporating varying levels of selection candidates generated via IVF and genomic selection. Bulls generated via IVF coupled with genomic selection then had to compete with a wider population of bulls generated from conventional matings into the final pool of bulls available for selection.

An initial pool of selection candidates was generated using 25 unrelated animals of each sex expressing a single polygenic normally distributed trait with a heritability (h^2) of 0.25. The burn in phase was simulated over 9 discrete generations of random mating between equal numbers of

males and females with known pedigree to generate an effective population size of 50 on average, before expanding out via factorial mating to 60,000 individuals in the 10th generation. True breeding values (TBV) in the first generation were drawn from a random normal distribution with a mean of zero and standard deviation equal to the square root of the heritability. For subsequent generations where parent information was available, the progeny TBV was calculated as the average of the parent TBV plus a Mendelian sampling term. Estimated breeding values (EBV) were simulated for the expanded population individuals as a trait correlated to their true breeding values using a Cholesky decomposition (Van Vleck and Gregory, 1992), with an accuracy of 0.8 simulated for genomic BVs.

Following the expansion stage, a genomic selection breeding scheme was simulated by selecting 50 sires and 1500 dams on EBV from the final pool of 60,000 individuals. The mating structure was weighted such that the top quintile of sires by EBV were randomly assigned the top 60% of the dams, the second quintile were randomly assigned to the next highest 20% of dams, and then the third, fourth and final quintile were assigned the remaining best ranked 10, 6 and 4% of dams respectively, to replicate a commercial industry structure. Each mating produced a single male offspring, resulting in a contribution of 1500 bull calf candidates to the final pool for selection.

Additional selection candidates resulting from IVF with prior prediction of genetic merit and selection at the pre-implantation stage were generated. The reproductive technologies processes were simulated using a series of random variates to assess the likelihood of a given cross between a selected male and female progressing through each stage from oocyte production through to survival of a semen producing bull. Top sires and dams were selected from the pool of candidates on EBV and mated using a factorial cross mating design. It was assumed that females could be flushed for oocytes multiple times, with each cross producing 10 oocytes.

Table 1. The base input parameters used to simulate the stages of the IVF process, including the unit for each input factor

IVF input parameters	Value	Unit
Oocytes	10.7	Per cow flushed
Viability rate of oocytes	0.9	Oocytes viable per oocytes recovered
IVP development rate	0.31	Blastocysts for testing per oocyte flushed
DNA tests	1	Per blastocyst for testing
Semen sexing rate	0.5	Male blastocysts per blastocyst for testing
Selection rate	0.05 to 1	Selected blastocysts/blastocyst tested
Mortality (post biopsy, cryopreservation)	0.1	Deaths per biopsied blastocyst
Cloning factor	4	Demi-embryos per embryo cloned
Cloning success rate	0.85	Surviving embryos per demi-embryo
Embryos implanted per recipient	1	
Embryo survival rate	0.2 to 0.4	Per embryo implanted
Calf survival rate	0.95	Per calf born
Acceptable bulls	1	Per viable calf

For each oocyte simulated, a series of random standard uniform variates were generated to simulate the likelihood that each oocyte is viable and passes the in-vitro production (IVP) stage, using the base parameters shown in Table 1. Genomic EBVs were generated for the remaining blastocysts assigned to be male using the equation below

$$GEBV = C_{[2,1]} \times TBV + C_{[2,2]} \times a, \text{ where } a \sim Norm(0,1),\ C = Chol\begin{bmatrix} 1 & \rho^2 \\ \rho^2 & \rho^2 \end{bmatrix}$$

where ρ^2 is the accuracy of genomic selection. Blastocysts were then selected prior to cloning at a specified rate using truncation selection.

The cloning of the embryos occurred after the biopsy and genomic selection stage, with up to 4 demi-embryos created per embryo cloned and a cloning success rate of 85%. Finally, random standard uniform variates were used for the likelihood of both embryo and calf survival, with the assumption that all surviving calves were acceptable for potential usage as a bull, and these bulls were added to the pool of conventional selection candidates. Each of these probabilities act as linear multipliers. The values shown in Table 1 for example, a single cow flushed would produce between 0.087 calves with a blastocyst pre-selection rate of 0.05 and 1.17 calves with a pre-selection rate of 1, where all blastocysts were retained.

Costs were assigned to each stage of the IVF process, then multiplied by the input parameters shown in table 1. If there was no pre-selection of blastocysts, the estimated cost to produce a single viable bull calf was $2,647 (NZ), of which 57% was attributed to rearing costs.

Scenarios were compared based on the top 50 bulls selected from the pool of 1500 selection candidates via truncation selection, and according to their additional costs attributable to the IVF process. The proportion of IVF bulls selected in the top 50 out of those generated was compared, along with the TBV superiority of the top 50 bulls. The pre-selection rate applied to the IVF blastocysts following DNA testing was varied between 0.05 and 1 (i.e. all blastocysts selected) and the number of cows flushed increased to maintain the same number of effective calves for each selection rate. The number of effective calves generated was varied between 2 and 36, with an embryo mortality rate of 0.2.

RESULTS AND DISCUSSION

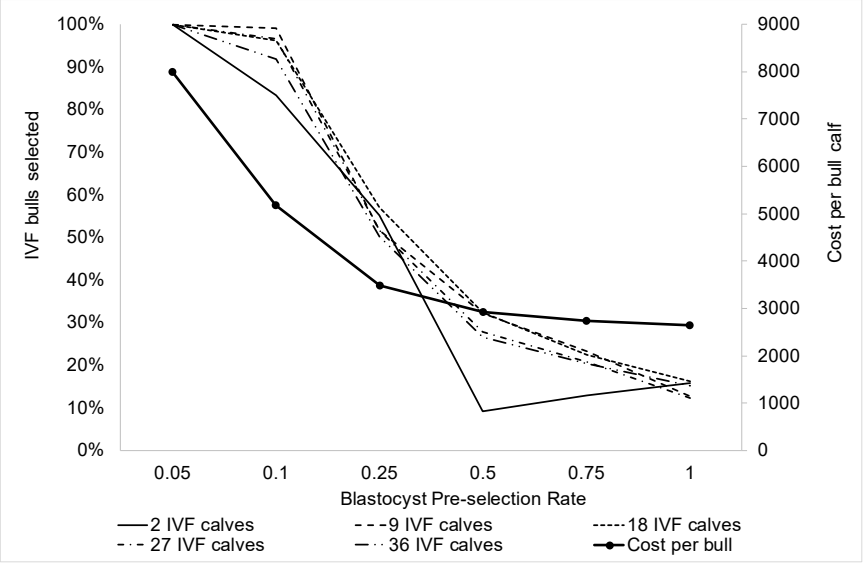

Figure 1. The average percentage of IVF bulls created that were selected in the top 50 as the blastocyst pre-selection rate increased from 0.05 to 1, with between 2 and 36 IVF calves created from the required number of cows flushed. The cost per bull calf for the given pre-selection rate is shown on the secondary axis

Figure 1 shows the average percentage of the IVF bulls generated that were selected in the top 50 as the pre-selection rate applied to blastocysts during the IVF process was increased from 0.05 to 1, with the cost per bull calf produced. When the top 5% of blastocysts were pre-selected for implantation using genomic selection, all bull calves created via IVF were selected in the top 50 candidates in all replicates, regardless of the number of calves created, at a cost of $7,990 per calf. The percentage selected dropped to between 50 and 60% with a pre-selection rate of 0.25 and a cost of $3490 per calf, with a further reduction to between 10 and 20% without any pre-selection of blastocysts. These results show that the pre-selection rate applied to blastocysts was the key variable, where a low selection rate of blastocysts would require a larger number of cows to be flushed and blastocysts biopsied for DNA testing, (e.g. 100 cows flushed to produce on average 9 IVF calves) but in turn this would reduce the rearing costs of those young bull candidates which would not have been of sufficiently high merit to be selected in the top 50 after pooling with the calves selected at birth.

Table 2 shows the mean TBV and EBV of the pool of 1500 young bull candidates, along with the non-IVF bull and IVF bulls and IVF bull calves selected in the top 50 for a blastocyst pre-selection rate of 0.05. The TBV of the IVF bull calves was significantly ($p<0.05$) higher than the non-IVF bulls with 9, 18 or 27 IVF calves produced, although this superiority was not observed with pre-selection rates higher than 0.1.

Table 2. The mean (standard deviation) TBV and EBV of all candidates for selection, the non-IVF bull calves and IVF bull calves in the top 50 selected, with a blastocyst pre-selection rate of 0.05

IVF calves produced	9 IVF Calves	18 IVF Calves	27 IVF Calves	36 IVF Calves
Cows Flushed	100	200	300	400
All Candidates TBV	0.86 (0.25)	0.84 (0.24)	0.84 (0.24)	0.84 (0.23)
All Candidates EBV	0.55 (0.16)	0.55 (0.15)	0.56 (0.15)	0.56 (0.15)
Non IVF bull TBV	1.23 (0.26)	1.21 (0.25)	1.22 (0.25)	1.22 (0.24)
Non IVF bull EBV	1.49 (0.17)	1.50 (0.15)	1.51 (0.15)	1.54 (0.16)
IVF bulls selected	9.12 (2.39)	17.56 (4.75)	26.72 (4.61)	36.92 (6.09)
IVF bull TBV	1.45 (0.30)	1.43 (0.34)	1.39 (0.28)	1.37 (0.30)
IVF Bull EBV	1.78 (0.19)	1.74 (0.21)	1.73 (0.20)	1.70 (0.19)

The utilisation of IVF technologies in combination with genomic selection at the blastocyst stage could be advantageous from a breeding company perspective, to carry out intensive selection on blastocysts prior to implantation, as it would reduce the rearing costs required to identify the top young bulls. While the cost of a large scale IVF program incorporating genomic selection may be prohibitive, the scenarios tested in this simulation project suggest that it could be used in combination with a more traditional genomic selection breeding scheme to increase the merit of the semen marketed from young bulls.

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