

# Effects of Different Methods for Determining the Number of Transferable Embryos on Genetic Gain and Inbreeding Coefficient in a Japanese Holstein MOET Breeding Population

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**ABSTRACT** : This study was conducted to examine the relationships between the methods used to determine the number of transferable embryos collected per flush and the estimated cumulative genetic improvements in the Japanese Holstein MOET breeding population. Cumulative genetic improvements were predicted by Monte Carlo simulation using three different determination methods (MODEL 1, MODEL 2, and MODEL 3), for calculating the number of embryos collected per flush. Moreover EBVs were estimated including or ignoring coefficients of inbreeding in MME. Inbreeding coefficients were also predicted. The number of transferable embryos was determined using normal, gamma, and Poisson distributions in MODEL 1, gamma and Poisson distributions in MODEL 2, and only the Poisson distribution in MODEL 3. The fitness of MODEL 2 in relation to field data from Hokkaido Japan was the best, and the results for MODEL3 indicated that this model is unsuitable for determining the number of transferable embryos. The largest cumulative genetic improvement (3.11) in the 10th generation was predicted by MODEL 3 and the smallest (2.83) by MODEL 2. Mean coefficients of correlation between the true and estimated breeding values were 0.738, 0.729, and 0.773 in MODELS 1, 2, and 3, respectively. It is suggested that the smallest genetic improvement in MODEL 2 resulted from the smallest correlation coefficient between the true and estimated breeding values. The differences in milk, fat, and protein yields between MODELS 2 and 3 were 182.0, 7.0, and 5.6 kg, respectively, in real units when each trait was independently selected. The inbreeding coefficient was the highest (0.374) in MODEL 2 and the lowest (0.357) in MODEL 3. The effects of different methods for determining the number of transferable embryos per flush on genetic improvements and inbreeding coefficients of the simulated populations were remarkable. The effects of including coefficients of inbreeding in MME, however, were unclear. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 5 : 597-602)

**Key Words** : Holstein, MOET, Transferable Embryos, Coefficients of Inbreeding, MME, Genetic Gain

## INTRODUCTION

Many evaluations, suggestions and practices for MOET (multiple ovulation and embryo transfer) have been reported (Nicholas and Smith, 1983; Ruane, 1988; Woolliams, 1989; Keller and Teepker, 1990; Teepker and Smith, 1990; Ruane, 1991a; Ruane, 1991b; Lohuis et al., 1993; Leitch et al., 1995). Prior to the use of a MOET breeding program, it is important to plan for and to evaluate the promising programs. MOET breeding programs are usually evaluated using genetic statistics (genetic gain, inbreeding coefficient, etc.) predicted by simulation. The number of transferable embryos collected per flush is an important factor with regard to the genetic gain of MOET breeding programs (Nicholas and Smith, 1983; Keller and Teepker, 1990; Leitch et al., 1995). Therefore, the quality of a simulation program depends heavily on the routine for generating the number of transferable embryos per flush. When this routine does not fully reflect the level of the MOET

technique in the experimental population, the prediction made by the simulation program is unreliable. Studies evaluating the use of MOET for the genetic improvement of ruminants have frequently assumed a fixed number of embryos per collection. Several studies have considered variable family size but have used hypothetical distributions (Villanueva et al., 1995). Asada and Terawaki (2000) have estimated suitable parameters and compared the adaptation of appropriate methods to the data regarding the number of transferable embryos collected per flush in the Japanese Holstein population.

It is expected that the rate of inbreeding will increase to very high levels over generations in MOET breeding populations. Including coefficients of inbreeding in the BLUP evaluation may have various effects on the response to selection.

The objectives of the present study were 1) to simulate the Holstein MOET breeding program in Japan using three different methods for generating the number of transferable embryos collected per flush and to examine the influence of the generation method on the genetic gain estimated by the simulation; and 2) to determine the effects of including coefficients of inbreeding in MME (mixed model equations) in an additive genetic model for genetic gain and the rate of inbreeding in the MOET breeding population.

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## MATERIALS AND METHODS

The conditions used for the simulation are presented in table 1. The object of the simulation was a Holstein population that consisted of 200 cattle in the base generation. Animals in the base population were assumed to be unrelated. The breeding program involved juvenile MOET, and the generation was discrete. The number of transferable embryos collected per flush was generated by three different methods developed by Villanueva et al. (1995). In MODEL 1, the number of transferable embryos collected per flush was generated from a negative binomial distribution. In practice, the number of transferable embryos collected per flush was generated using the Poisson distribution with parameter  $\lambda$ . Lambda ( $\lambda$ ) was generated using a gamma distribution with parameter  $\beta$ . Log  $\beta$  was derived from a standardized normal distribution. In MODEL 2 a normal distribution was not used and the two parameters ( $\alpha$  and  $\beta$ ) of the gamma distribution were fixed at the same values. In MODEL 3 the number of transferable embryos collected per flush was generated using only the Poisson distribution. The parameter values for the different models used to generate the number of transferable embryos collected per flush were determined according to Asada and Terawaki (2000). Parameters for the normal and gamma distributions ( $\mu$ ,  $\sigma^2$ , and  $\alpha$ ) were set at 4.5, 0.1, and 1.0 in MODEL 1. The parameters ( $\alpha$  and  $\beta$ ) of the gamma distribution in MODEL 2 were 1.0 and 4.4. Parameter ( $\lambda$ ) of the Poisson distribution was set at 5.7 in MODEL 3. MODEL 1 could take repeated treatment into account (Woolliams et al., 1995, Villanueva et al., 1995). But, repeated treatment of an animal was not taken into account in the present study, as only a few animals were treated repeatedly. Ten males and 30 females were selected for obtaining estimated breeding values (EBV) using

an animal model, BLUP, at each generation. EBV were estimated by two different ways. Coefficients of inbreeding were included in the BLUP evaluation in one way and ignored in the other. Only one animal out of full-sib brothers was selected. Matings were randomly carried out between males and females selected on EBVs. Eighty-five matings per generation were carried out in MODELS 1 and 2, and 65 were carried out in MODEL 3. These numbers of matings per generation were used in order to maintain the population size at as close as possible to 200 animals. The true breeding values of the offspring were generated by adding the mean of their parents true breeding values and the Mendelian sampling term. The conception rate was 50 %. A lactation trait was selected, and the heritability of the selected trait was 0.3. Simulations were repeated 50 times per generating method. Field data from 143 MOET treatments of 96 cows were collected in Hokkaido, Japan.

## RESULTS AND DISCUSSION

Table 2 provides statistics regarding the number of transferable embryos collected per flush resulting from simulations using three different generation methods. In addition, the field data for multiple ovulation and embryo transfer in Hokkaido, Japan is shown. The mean number of transferable embryos collected per flush was the largest (5.69) in MODEL 3 and smallest (4.31) in MODEL 1. However, the mean number of transferable embryos collected per flush according to the field data was 3.78. This means that the number in the field data was smaller than the means of the three simulations. The frequencies of treatments which collected no transferable embryos were the highest, except in MODEL 3. The probability of treatment collecting no transferable embryos on total treatments frequency was highest (28.7 %) for the field data and this was close to the probability (20.0 %) in MODEL 1. MODEL 2 fitted the field data best and this was followed by MODEL 1. The chi-square value was highest in MODEL 3, indicating that MODEL 3 is ineffective in determining the number of transferable embryos collected per flush in Hokkaido, Japan. The mean number of transferable embryos collected per flush and the probability of treatments collecting no transferable embryos in MODEL 1 were closer to those for the field data than they were to those for MODEL 2. These results indicate that MODEL 1 gives values closest to those for the field data among the three generating methods. However, chi-square values indicated that MODEL 2 had the best fit with the field data. The frequency distribution of the number of transferable embryos collected per flush in the field data was low in groups with two and three transferable embryos, and high in groups with 4, 5, 6, 10, and 11 transferable embryos (Asada and Terawaki

**Table 1.** Conditions of simulation

Population	Holstein cattle
Number of cattle in base population	200 ( $\sigma : \phi = 1:1$ )
Method for generating number of transferable embryos per treatment	MODEL 1, MODEL 2, MODEL 3*
Number of mating per generation	85, 85, 65
Conception rate	50%
Evaluation method for genetic performance	BLUP with animal model
Selected trait	A lactation trait
Heritability	0.3

\* Villanueva et al. (1995), and Asada and Terawaki (2000).

**Table 2.** Comparison among methods for generating the number of transferable embryos per flush

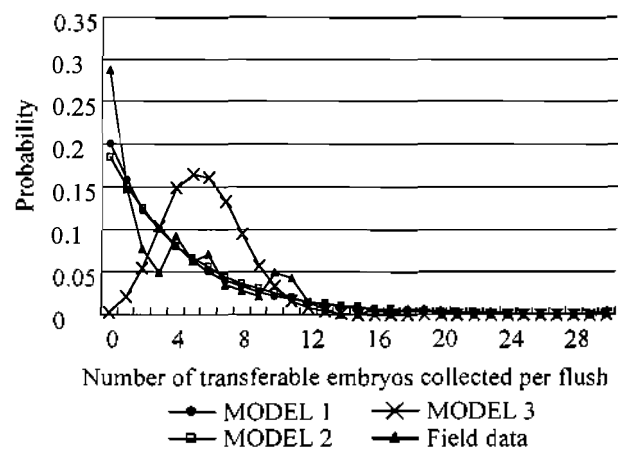
Item	Methods of generation			Field data
	MODEL 1	MODEL 2	MODEL 3	
Mean number of transferable embryos per flush	4.31	4.39	5.69	3.78
Number of transferable embryos occurring at the highest treatment frequency	0	0	5	0
Probability of treatments having the number of transferable embryos indicated in the upper line	20.0	18.5	16.4	28.7
Fitness for field data ( $\chi^2$ )	30.5	29.5	4056.8	-

2000). The lack of smoothness in the distribution of the number of transferable embryos per flush in the field data may cause the discrepancy in the chi-square values, the mean, and the probability of treatments with no transferable embryos per flush for MODELS 1 and 2. Woolliams et al. (1995) and Villanueva et al. (1995) have discussed the merits of MODEL 1, in that it can take into account donor repeatability in relation to the number of transferable embryos per flush. However, the present data included few cows who underwent repeated MOET treatments. Therefore, it may be difficult to determine the merits of MODEL 1 in the present study. It is also clear from some of the present results that the fitness of MODEL 3 is significantly inferior to those of the other models considered here and this is consistent with the results of Asada and Terawaki (2000).

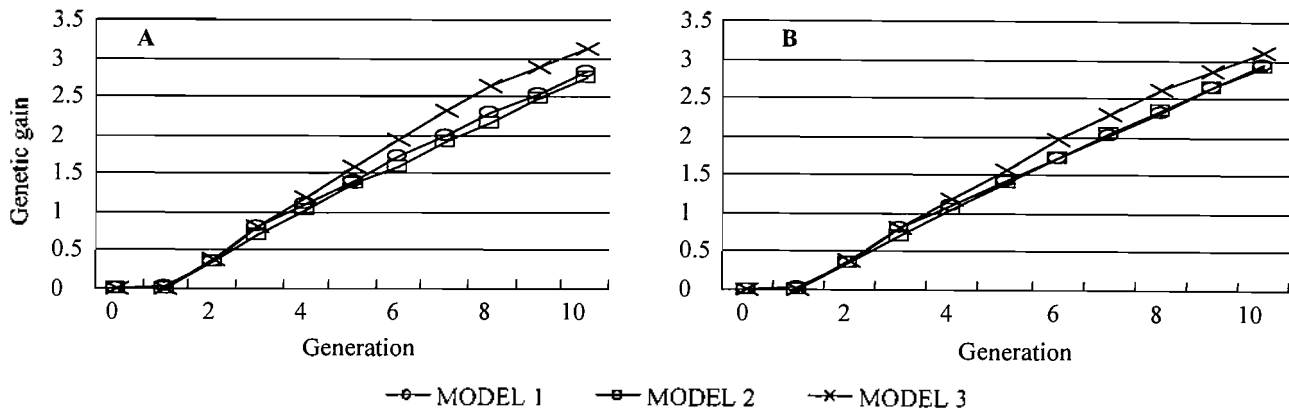
The probability distributions of the number of transferable embryos collected per flush generated in the simulations are shown in figure 1. Figure 1 also provides the distribution for the field data. The probability distributions for MODELS 1 and 2 are similar to that of the field data, indicating that MODELS 1 and 2 are adaptable enough with regards to the field data to be used in simulations for estimating the genetic gain of the MOET breeding program. The probability of having treatments resulting in no transferable embryos in MODELS 1 and 2 was lower than in the field data, and the probability of treatments resulting in two and three embryos was higher. It is therefore necessary to improve the fitness of MODELS 1 and 2 in cases with a small number of transferable embryos collected per flush.

Genetic improvements based on the use of three different generation methods are illustrated in figure 2. Graph A in figure 2 represents a case that takes into account the inbreeding of animals in MME, and graph B ignores the inbreeding of animals. Genetic improvements remarkably increased in the second generation in all models and differences in genetic gains among the models were not visible until the third generation. Thereafter, the differences among the three models became large. In particular, the genetic

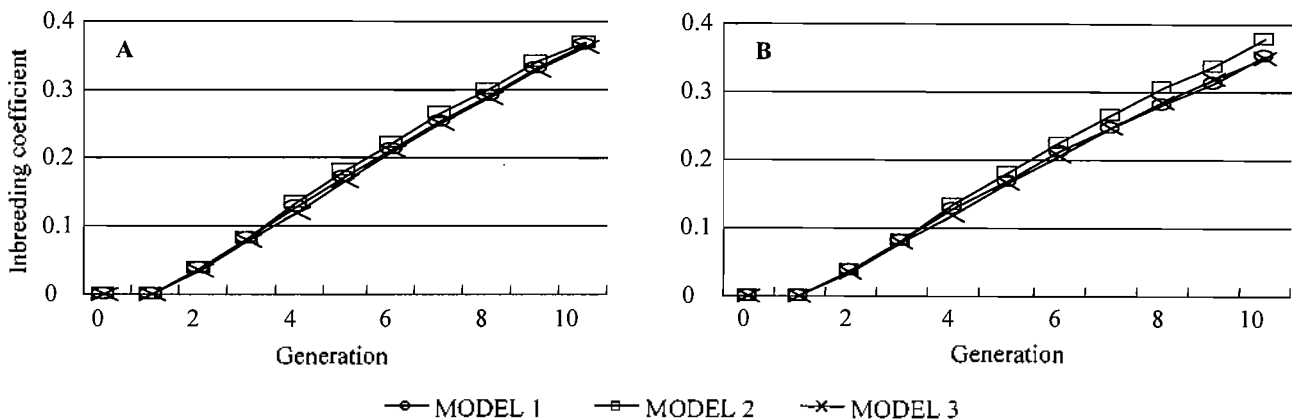
gain estimated using MODEL 3 was obviously different from those of the other two models. On the whole, genetic improvements over multiple generations were the largest when estimated using MODEL 3 and the smallest when using MODEL 2. The differences between MODELS 1 and 2 were not distinct. The similarity in the distributions of the number of transferable embryos collected per flush in MODELS 1 and 2 (figure 1) resulted in similar trends in the genetic gains estimated using these two models. The effects on genetic gains of including coefficients of inbreeding in MME for an animal model were unclear. When the inbreeding coefficients of the animals were ignored in MME, larger genetic gains were estimated in MODELS 1 and 2 which agreed with the results reported by Mehrabani-yeganeh et al. (2000). However, the results obtained with MODEL 3 differed from those obtained with MODELS 1 and 2. The coefficients of correlation between EBV and true BV were 0.732, 0.717, and 0.775 in MODELS 1, 2, and 3, respectively, when coefficients of inbreeding were considered in constructing the inverse of the relationship matrix. When the inbreeding of the



**Figure 1.** Probability distributions of number of transferable embryos collected per flush generated using three methods (MODELS 1, 2 and 3), and from field data



**Figure 2.** Mean genetic gains of simulated population estimated with three methods to generate number of transferable embryos including (graph A) or ignoring (graph B) coefficients of inbreeding in MME



**Figure 3.** Mean inbreeding coefficients of simulated population estimated with three methods to generate number of transferable embryos including (graph A) or ignoring (graph B) coefficients of inbreeding in MME

animals was ignored, the coefficients of correlation were 0.744, 0.740, and 0.770 in MODELS 1, 2, and 3, respectively. The largest genetic gain of MODEL 3 then results from the highest accuracy of selection.

Figure 3 shows the means of the inbreeding coefficients estimated by the three generation methods. The mean coefficients of inbreeding were the highest in MODEL 2 and the lowest in MODEL 3 irrespective of including or ignoring coefficients of inbreeding in MME. But the differences with regard to inbreeding among the generation methods were not remarkable. The smallest amount of inbreeding has also been estimated for MODEL 3 by Villanueva et al. (1995). Villanueva et al. (1995) estimated the highest level of inbreeding for MODEL 1, which takes into account donor repeatability in determining the number of transferable embryos collected per flush. In the present study, however, the coefficient of inbreeding for MODEL 1 was second highest because donor repeatability was ignored.

Analysis of variance was carried out in order to examine the influences of the various methods in generating the number of transferable embryos per flush and including coefficients of inbreeding in MME on genetic gain and the inbreeding coefficient of a simulated population. Analysis by the GLM procedure (SAS, 1988) indicated that methods significantly influenced genetic gains and inbreeding coefficients. Table 3 shows the least-square means of genetic gain and inbreeding coefficients for the generation methods. Genetic gains in MODEL 3 were considerably larger than those in MODELS 1 and 2 from generation 6 to 8. The differences in the inbreeding coefficients among the generation methods were not clear in comparison with the genetic gains.

Least-square means of genetic gain in the 10th generation expressed in real units from the genetic standard units are shown in table 4. The genetic standards of milk, fat, and protein yields were assumed to be 650, 25, and 20 kg, respectively.

**Table 3.** Least-square means of genetic gain and inbreeding coefficient for methods to generate the number of transferable embryos per flush

Generation	Genetic gain			Inbreeding coefficient		
	Model 1 <sup>1</sup>	Model 2 <sup>1</sup>	Model 3 <sup>1</sup>	Model 1 <sup>1</sup>	Model 2 <sup>1</sup>	Model 3 <sup>1</sup>
1	0.04 <sup>aA</sup>	0.01 <sup>aA</sup>	0.01 <sup>aA</sup>	0.0 <sup>aA</sup>	0.0 <sup>aA</sup>	0.0 <sup>aA</sup>
2	0.34 <sup>aA</sup>	0.34 <sup>aA</sup>	0.37 <sup>aA</sup>	0.037 <sup>aA</sup>	0.037 <sup>aA</sup>	0.033 <sup>bA</sup>
3	0.80 <sup>abA</sup>	0.70 <sup>bA</sup>	0.82 <sup>aA</sup>	0.080 <sup>aA</sup>	0.082 <sup>aA</sup>	0.079 <sup>aA</sup>
4	1.11 <sup>abA</sup>	1.03 <sup>bA</sup>	1.18 <sup>aA</sup>	0.127 <sup>abAB</sup>	0.134 <sup>aA</sup>	0.118 <sup>bB</sup>
5	1.41 <sup>bA</sup>	1.39 <sup>bA</sup>	1.58 <sup>aA</sup>	0.171 <sup>abAB</sup>	0.180 <sup>aA</sup>	0.165 <sup>bB</sup>
6	1.72 <sup>bB</sup>	1.65 <sup>bB</sup>	1.96 <sup>aA</sup>	0.212 <sup>abA</sup>	0.223 <sup>aA</sup>	0.208 <sup>bA</sup>
7	2.01 <sup>bB</sup>	1.97 <sup>bB</sup>	2.30 <sup>aA</sup>	0.250 <sup>bA</sup>	0.266 <sup>aA</sup>	0.248 <sup>bA</sup>
8	2.31 <sup>bB</sup>	2.25 <sup>bB</sup>	2.62 <sup>aA</sup>	0.287 <sup>bA</sup>	0.303 <sup>aA</sup>	0.286 <sup>bA</sup>
9	2.59 <sup>bA</sup>	2.55 <sup>bB</sup>	2.87 <sup>aA</sup>	0.323 <sup>bA</sup>	0.339 <sup>aA</sup>	0.323 <sup>bA</sup>
10	2.87 <sup>bA</sup>	2.83 <sup>bA</sup>	3.11 <sup>aA</sup>	0.360 <sup>abA</sup>	0.374 <sup>aA</sup>	0.357 <sup>bA</sup>

<sup>1</sup> Method for generating the number of transferable embryos per flush; <sup>aA</sup> Values on the same line without a common superscript differ ( $p < 0.05$ ); <sup>ab</sup> Values on the same line without a common superscript differ ( $p < 0.01$ ).

Differences in cumulative genetic gain between MODELS 3 and 2 were 182, 7, and 6 kg for milk, fat, and protein yields.

### CONCLUSION

MODELS 1 and 2 were found to be adaptative enough to estimate cumulative genetic gain in a MOET breeding population of Japanese Holsteins. In contrast, MODEL 3 was found to be unsuitable for

use with the MOET breeding population in Japan. These results are strictly for the Japanese Holstein population and it is important to determine the best model for determining the number of transferable embryos to be collected per flush for a specific population. Significant differences in cumulative genetic gain among the generation methods were recognized, differences that apparently result from the accuracy of selection. Including coefficients of inbreeding in MME appeared to have no influence on the genetic gain and inbreeding coefficient of the simulated population in this study.

**Table 4.** Means of milk, fat, and protein yields<sup>1</sup> in the 10th generation predicted by simulation, and differences between methods when each trait was independently selected

Milk yield			
Method <sup>4</sup>	MODEL 1	MODEL 2	MODEL 3
MODEL 1	1865.5 <sup>2</sup>		
MODEL 2	-26.0 <sup>3</sup>	1839.2	
MODEL 3	156.0	18.2	2021.5
Fat yield			
Method	MODEL 1	MODEL 2	MODEL 3
MODEL 1	71.8		
MODEL 2	-1.0	70.8	
MODEL 3	6.0	7.0	77.8
Protein yield			
Method	MODEL 1	MODEL 2	MODEL 3
MODEL 1	57.4		
MODEL 2	-0.8	56.6	
MODEL 3	4.8	5.6	62.2

<sup>1</sup> Assumed genetic standard deviation to be 650, 25, and 20 kg for milk, fat, and protein yields.

<sup>2</sup> Diagonal figures indicate means.

<sup>3</sup> Non-diagonal figures indicate differences between models.

<sup>4</sup> Method for generating number of transferable embryos per flush.

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