Genetic Analysis of Traits Affecting the Success of Embryo Transfer in Dairy Cattle

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ABSTRACT

The primary aim of this study was to estimate variance components for traits related to embryo transfer (ET) by applying generalized linear mixed models (GLMM) for different distributions of traits (normal, binomial, and Poisson) in a synergistic context. Synergistic models were originally developed for traits affected by several genotypes, denoted as maternal, paternal, and direct effects. In the case of ET, the number of flushed ova (FO) only depends on a donor's maternal genetic effect, whereas paternal fertility must be considered for other embryo survival traits, such as the number of transferable embryos (TE), the number of degenerated embryos (DE), the number of unfertilized oocytes (UO), and the percentage of transferable embryos (PTE). Data for these traits were obtained from 4,196 flushes of 2,489 Holstein cows within 4 regions of northwest Germany from January 1998 through October 2004. Estimates of maternal heritability were 0.231 for FO, 0.096 for TE, 0.021 for DE, 0.135 for UO, and 0.099 for PTE, whereas the relative genetic impact of the paternal component was near zero. Estimates of the genetic correlations between the maternal and the paternal component were slightly negative, indicating a genetic antagonism. For the analysis of pregnancy after ET, 8,239 transfers to 6,819 different Holstein-Friesian recipients were considered by applying threshold methodology. The direct heritability for pregnancy in the recipient after ET was 0.056. The relative genetic impact of maternal and paternal components on pregnancy of recipients describing a donor's and a sire's ability to produce viable embryos was below 1%. The genetic correlations of the direct effect of the recipient with the sire of embryos (paternal effect) and the donor cow (maternal effect) for pregnancy after ET were -0.32 and -0.14 , respectively. With the exception of FO and PTE (-0.17), estimates of genetic correlations among

traits for the maternal site were distinctly positive, especially between FO and TE (0.74). Based on this high genetic correlation and due to the higher heritability for FO, indirect selection on FO will increase selection response in TE by about 22% compared with direct selection on TE. The negative genetic correlation of -0.27 between TE and lactation milk yield indicates the need for development of an index for bull dams in multiple ovulation and embryo transfer (MOET) breeding schemes combining production as well as traits related to ET.

Key words: embryo transfer, synergistic groups, generalized linear mixed model, genetic parameter

INTRODUCTION

Nicholas and Smith (1983) introduced the application of a nucleus herd program based on multiple ovulation and embryo transfer (MOET) for increased genetic response to selection in dairy cattle. Subsequent studies in the 1980s and 1990s (e.g., Colleau, 1991) have followed, and the results revealed a potential of 10 to 20% additional genetic gain compared with traditional breeding programs. Gains are mainly due to the reduction of generation intervals. Consequently, several dairy cattle breeding programs for the Holstein-Friesian breed in Germany as summarized by König and Swalve (2003) have included the ideas of Nicholas and Smith (1983) and established MOET breeding schemes to produce as many offspring of elite cows as possible. This was mainly accomplished in combination with an expensive centralized test on-station for potential bull dams to avoid biases in EBV due to preferential treatment (Kuhn et al., 1994) and due to the effect of heterogeneous variances in different environments on estimated breeding values of cows (e.g., Garrick and Van Vleck, 1987). However, the goal to produce at least 5 full sisters and 5 full brothers through the application of embryo transfer (ET) with the intention of replacing progeny testing and reducing generation intervals often failed.

As noted by König and Swalve (2003) and König et al. (2007), the superiority of MOET breeding programs

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will mainly depend on the number of progeny of superior cows, and therefore depend on the success rate of ET. In addition to the improvement of technical methods associated with the ET biotechnology (e.g., Hasler, 1992) and the improvement of environmental conditions for donors and recipients (e.g., Kafi and McGowan, 1997), selection for characteristics related to ET can also contribute to more offspring per donor.

Genetic evaluations require accurate estimates of parameters. However, for traits related to ET, appropriate data are rare, and previous genetic studies were based on breeds other than Holstein (Liboriussen et al., 1995), were accomplished in different populations (Benyei et al., 2004), or are somewhat outdated (Preisinger et al., 1990). Furthermore, recent developments in statistical methods allowing application of a generalized linear mixed model (GLMM) technique (Schall, 1991) can be used to analyze ET data with appropriate distributions, such as normal, binomial, and Poisson. In the case of ET, the complexity of several traits and of several genotypes has to be taken into account, and knowledge about their variance and covariance components for maternal and paternal effects is of basic concern and was not fully considered in previous studies. From the genetic point of view, the number of flushed ova describes a cow's own performance and only depends on the donor's genetic effect, whereas for traits describing embryonic survival, the paternal effect also has to be taken into account. The final pregnancy rate of embryos is affected by the genotype of the recipient, the genotype of the donor, and the genotype of the sire of embryos. Willham (1963) defined these traits depending on groups of animals representing direct, maternal, and paternal effects, as synergistic traits. The theoretical background of statistical models for evaluating synergistic traits in the case of fertility was carefully evaluated by Haussmann and Heinkel (1989). The increase of inbreeding in the German Holstein population (and thus, the close additive genetic relationships among animals and especially between bull dams and bull sires; König and Simianer, 2006) enables reliable estimates of the covariances among genetic groups involved in a synergistic model for ET.

The objectives of this study were to estimate variance and covariance components for the ovulation rate, traits describing embryonic survival, and pregnancy after ET in a synergistic context and to apply generalized linear mixed models with appropriate link functions for various distributions of the traits studied. Results give detailed information on the interaction of different genotypes for different traits describing fertility in dairy cattle. As a further extension of this analysis, traits related to ET were correlated with other production traits of superovulated cows. Knowledge of variance

Table 1. Descriptive statistics of analyzed traits of embryo donor cows

Trait	Mean	SD	Minimum	Maximum
Flushed ova, n	8.52	6.91	0	52
Transferable embryos, n	4.84	5.00	0	33
Degenerated embryos, n	0.81	2.25	0	29
Unfertilized oocytes, n	2.64	4.61	0	34
PTE, ¹ %	48.43	10.62	0	100
305-d milk yield, kg	9,962.78	1,507.60	8,705.19	14,831.77
Fat, %	4.16	0.48	3.51	5.59
Protein, %	3.44	0.19	2.63	4.14
SCS	2.39	1.11	0.04	7.28

¹PTE = percentage of transferable embryos = no. of transferable embryos/no. of flushed ova.

and covariance components of all these traits can be used to develop an index or a complex breeding goal for potential bull dams and ultimately contribute to more success of existing MOET breeding schemes.

MATERIALS AND METHODS

Data

Data related to ET were obtained from 4,196 flushings of 2,489 Holstein cows within 4 regions corresponding to 4 different breeding organizations (A, B, C, and D) in northwest Germany from January 1998 through October 2004. Traits of interest were the number of flushed ova (FO), the number of transferable embryos (TE), the number of degenerated embryos (DE), the number of unfertilized oocytes (UO), and the percentage of transferable embryos (PTE; $PTE = TE \times 100 / FO$). Production traits were 305-d lactation yields for milk (kg), fat percentage, protein percentage, and SCC of all donor cows for the respective flushing year. Somatic cell count was log-transformed into SCS to achieve normality and homogeneity of variances by applying the formula by Ali and Shook (1980).

Donor cows were generally intensively selected elite cows for production (Table 1) and conformation traits and were heavily used as bull dams. Hence, the main intention of ET was to ensure at least one male offspring as a young sire for the progeny testing program, but female progeny were preferred when selecting the next generation of future donor cows. Breeding organization A has implemented these ideas of an open MOET breeding scheme for nearly 20 yr (Kandzi, 1988). The cycle of this breeding program is explained in Figure 1. On-station, bull dams were selected primarily for protein yield, type classification, and SCC, but currently there is no selection pressure on traits related to ET.

A total of 8,239 embryos were transferred to 6,819 different purebred Holstein-Friesian recipients kept in herds contracted by the respective breeding organiza-

Figure 1. Structure of a multiple ovulation embryo transfer (ET) breeding program for one German breeding organization as developed by Kandzi (1988).

tion. Binary coded status of pregnancy of recipients 8 wk after ET was based on information from rectal palpation done by veterinarians. Phenotypic means, standard deviations, minima, and maxima for all traits are given in Table 1. Pedigrees of donor cows, service sires, and recipients were available back to base animals born in 1940.

Statistical Models

The general terminology used in this paper in the context of synergistic models is depicted in Figure 2.

Figure 2. Structure for a synergistic model in the case of embryo transfer. Arrows indicate possible or certain coefficients of relationship = a .

Maternal effect is the impact of the genetic mother (donor) of ova or embryos, and the paternal effect is the impact of the genetic father (sire) of the embryos. When evaluating genetic components, the number of FO only depends on a donor's maternal genetic effect, whereas for traits describing embryonic survival (in this study TE, DE, UO, and PTE), the paternal effect has to be taken into account. Hence, for TE, DE, UO, and PTE, genetic covariances between maternal and paternal effects can be estimated. The term "direct effect" is used in this paper only with regard to pregnancy after ET, and it describes the genetic ability of recipients to become pregnant. The maternal and paternal component in the case of pregnancy is the donor's and the sire's contribution, respectively, to produce viable embryos.

The maternal variance component for FO as well as maternal and paternal variance and covariance components for TE, DE, UO, and PTE were estimated using univariate animal models for REML and applying the ASREML package (Gilmour et al., 1998). The genetic correlations for all possible combinations of these traits were estimated for the maternal component of FO, TE, DE, UO, and PTE, and the various production traits using bivariate animal models. In a bivariate analysis for estimating genetic correlations between 2 categorical traits, ASREML treats 1 of these traits in the linear sense. However, Vinson and Kluwer (1976) have shown that the genetic correlation computed from multinomial or binomial phenotypes of related animals is equal to normally distributed variables, and vice versa. This was recently shown by Mielenz et al. (2005) when analyzing genetic parameters for mortality in laying hens.

For pregnancy of recipients after ET, a univariate animal model was used to estimate the covariance among involved genetic groups for maternal, paternal, and direct genetic effects.

The ASREML program allows specification of both the distribution of the traits and the application of a GLMM analysis through a link function. The traits FO, TE, DE, and UO are count variables following a Poisson distribution (Figure 3). The link function f_i between the linear predictor η_i and the observations y_i used for these count data was a log link defined as $f_i = \log_e(\eta_i)$. Data measured on the percentage scale (PTE) were transformed by the arc-sine transformation (Sokal and Rohlf, 1995) to achieve homogeneity of error variance and then analyzed in a GLMM applying an identity link function. The probit link, modeling the probability $[P(y = 1)]$ that a recipient is pregnant after ET is given by $f_i = \Phi^{-1}[\eta_i]$ where Φ^{-1} is the inverse normal cumulative density function. In this probit model, identical to a threshold liability model (Gianola, 1982; Gianola and Foulley, 1983), it is assumed that an underlying continuous variable, liability l_i , exists such that the observed binary

Figure 3. Distribution for flushed ova (black solid line), transferable embryos (gray solid line), and degenerated embryos (dotted line).

variable y_i takes a value of 1 if l_i is larger than a fixed threshold, and 0 otherwise. For calculating heritabilities in the GLMM Poisson-log model and in the threshold model, the residual variance was fixed to a value of 1.

Due to the different genetic groups involved in the phenotypic expression of the traits, different structures of the (co)variance matrices for random effects have to be applied. For FO, the model 1 for univariate analyses in matrix notation was

$$y = Xb + Zd + Spd + e, \quad [1]$$

where y = vector of observations, b = vector of the fixed breeding organization-year-season effect, d = vector of random genetic effects of the donor, pd = vector of permanent environmental effects of the donor, e = vector of random residual effects, and X , Z , and S , are the incidence matrices relating records to fixed and random effect. It is assumed that

$$\text{var} \begin{bmatrix} u \\ pd \\ e \end{bmatrix} = \begin{bmatrix} gA & 0 & 0 \\ 0 & I\sigma_{pd}^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix},$$

where g is the additive genetic variance for the maternal effect of the donor, A is the numerator relationship matrix, σ_{pd}^2 is the variance due to permanent environmental effects of the donor, σ_e^2 is the residual error variance, and I represents the identity matrix. Model 2 for TE, DE, UO, and PTE was

$$y = Xb + Zd + Spd + Ws + Vps + e, \quad [2]$$

where s and ps are the vectors of random genetic and random permanent environmental effects of the sire and other effects are the same as in model [1]. The corresponding matrix of variances and covariances for random effects, similar to an animal model for a maternal trait (Quaas and Pollak, 1980), was

$$\text{var} \begin{bmatrix} d \\ s \\ pd \\ ps \\ e \end{bmatrix} = \begin{bmatrix} g_{11}A & g_{12}A & 0 & 0 & 0 \\ g_{21}A & g_{22}A & 0 & 0 & 0 \\ 0 & 0 & I\sigma_{pd}^2 & 0 & 0 \\ 0 & 0 & 0 & I\sigma_{ps}^2 & 0 \\ 0 & 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix},$$

where g_{11} is the additive genetic variance for the maternal effect of the donor, g_{22} is the additive genetic variance for the paternal effect of the sire, and g_{12} is the additive genetic covariance between maternal and paternal effects. The variances due to permanent environmental effects of the donor and the sire are σ_{pd}^2 and σ_{ps}^2 , respectively.

For pregnancy after ET, the statistical model was extended to the direct genetic effects of the recipient. The model [3a] was as follows:

$$y = Xb + Mr + Npr + Zd + Ws + e, \quad [3a]$$

where r and pr are the vectors of random genetic and random permanent environmental effects of the recipient.

ent, and M and N are the corresponding incidence matrices. The additive genetic effects of the donor, vector d , as well as the additive genetic effects of the sire of embryos, vector s , describe the genetic component of embryo vitality. All genetic effects can be linked based on the relationship matrix, which allows the estimation of genetic covariances between the genetic groups involved. In the case of pregnancy after ET, the vector b included the fixed breeding organization-year-season effect, the status of transferred embryos (58.3% fresh, 41.7% frozen), quality classes 1 to 3 of embryos according to guidelines of the International Embryo Transfer Society (IETS; Stringfellow and Seidel, 1998), and embryo development according to IETS (3 different classes of embryo development).

Another, more straightforward alternative is to simplify model [3a] and to replace the sire and donor genetic terms by the genetic effect of the embryo. The model [3b] was

$$y = Xb + Mr + Npr + Qu + e, \quad [3b]$$

where vector u includes the additive genetic effect of the embryo and Q is the corresponding incidence matrix. All other effects are identical to those in model [3a].

RESULTS AND DISCUSSION

Variance Components for Genetic Groups Within Traits

Most investigations dealing with genetic effects on traits related to ET, such as FO or TE, have focused on breed differences (e.g., Herrler et al., 1991; Estrada et al., 1998); differences between breeds of donor cows were generally significant. However, estimates of genetic parameters for these traits within breeds and utilizing relatively large datasets including more than 1,000 donor cows are restricted to a few publications. Heritabilities for maternal, paternal, and direct effects as well as the relative impact of the permanent environmental components for different traits affecting the success of ET found in the present study are summarized in Table 2. Maternal heritabilities were 0.231 for FO, 0.096 for TE, 0.021 for DE, 0.135 for UO, and 0.099 for PTE. Variance component estimation for FO and TE was done in some previous investigations (Liboriussen et al., 1995; Tonhati et al., 1999), and results were in the same range despite major differences in statistical models. A substantially higher maternal heritability of 0.59 for TE was only found by Piexoto et al. (2004) for ET results in Nellore cattle.

The relative impact of the permanent environmental component of the donor was substantial and in the

range of 0.06 to 0.16 for the investigated traits, resulting in repeatabilities of 0.35 and 0.25 for FO and TE, respectively. Similar to the moderate maternal genetic impact of the donor on the number of FO, TE, and PTE, permanent environmental effects of the same magnitude due to special feeding or management strategies or due to a cow's individual reaction to hormone supplies can be anticipated when considering the ultimate success in ET. In contrast, the paternal heritability describing a sire's ability to fertilize flushed ova was quite low. The relative genetic impact of the paternal component was 0.012, 0.017, 0.027, and 0.014 for TE, DE, UO and PTE, respectively and is therefore similar to results of previous studies (e.g., Preisinger et al., 1990). The paternal permanent environmental component for these traits was also near zero. An explanation could be that high-quality semen is generally used for inseminations during ET. However, Bosselmann et al. (2005) found pronounced differences in the number of TE on the phenotypic scale for different service sires within flushing years, but these differences seemed to be associated with nongenetic effects.

The direct heritability for the recipient to become pregnant after ET was 0.056 (Table 2) and is therefore in the general range for nonreturn rates in dairy cattle reported in the literature (e.g., Jamrozik et al., 2005). A small fraction (17.2%) of all recipients was used for repeated embryo transfers and the permanent environmental component was small (1.7%). Interpreting this result, the preselection of recipients by veterinarians according to a recipient's fertility status must be taken into account. The relative genetic impact of maternal and paternal components on pregnancy of recipients describing a donor's and a sire's ability to produce viable embryos was less than 1%. The relative impact at the total variance for pregnancy was only 0.8% for the maternal component and 0.6% for the paternal component. However, in general, the poorest embryos were discarded before the transfer, explaining the minor importance of genetic parents of embryos when discussing fertility of recipients. The alternative model [3b] revealed a similar heritability for the recipient (0.058), and the relative importance of embryo vitality for pregnancy after ET was 1.5%. The more robust model [3b] led to higher additive genetic variances for the embryo effect; even standard errors for genetic correlations between synergistic groups were less than results obtained from model [3a] (Figure 4).

Genetic correlations between the maternal and paternal components within traits were negative, but near zero. Estimates for genetic correlations were -0.11 (± 0.139) for TE, -0.04 (± 0.127) for DE, -0.10 (± 0.132) for UO, and -0.08 (± 0.130) for PTE. Thus, selection for the maternal component for TE will tend to leave sire

Table 2. Relative genetic impact of the donor (h_d^2), the service sire (h_s^2), the recipient (h_r^2), and the embryo (h_e^2) for various traits related to embryo transfer (ET) and relative impact of respective permanent environmental components σ_{pd}^2 , σ_{ps}^2 , and σ_{pr}^2 for analyzed traits

Trait	Heritabilities				Permanent environment		
	$h_d^2 \pm \text{SE}$	$h_s^2 \pm \text{SE}$	$h_e^2 \pm \text{SE}$	$h_r^2 \pm \text{SE}$	$\sigma_{pd}^2/\sigma_p^2 \pm \text{SE}$	$\sigma_{ps}^2/\sigma_p^2 \pm \text{SE}$	$\sigma_{pr}^2/\sigma_p^2 \pm \text{SE}$
Flushed ova, n	0.231 $\pm$ 0.091	—	—	—	0.114 $\pm$ 0.043	—	—
Transferable embryos, n	0.096 $\pm$ 0.087	0.012 $\pm$ 0.075	—	—	0.153 $\pm$ 0.048	0.002 $\pm$ 0.049	—
Degenerated embryos, n	0.021 $\pm$ 0.081	0.017 $\pm$ 0.077	—	—	0.060 $\pm$ 0.047	0.001 $\pm$ 0.040	—
Unfertilized oocytes, n	0.135 $\pm$ 0.090	0.027 $\pm$ 0.082	—	—	0.067 $\pm$ 0.044	0.001 $\pm$ 0.041	—
PTE, ¹ %	0.099 $\pm$ 0.086	0.014 $\pm$ 0.075	—	—	0.161 $\pm$ 0.055	0.002 $\pm$ 0.048	—
Pregnancy after ET							
Model [3a] ²	0.008 $\pm$ 0.007	0.006 $\pm$ 0.002	—	0.056 $\pm$ 0.026	—	—	0.017 $\pm$ 0.014
Model [3b] ²	—	—	0.015 $\pm$ 0.002	0.058 $\pm$ 0.025	—	—	0.018 $\pm$ 0.013

¹PTE = percentage of transferable embryos = no. of transferable embryos/no. of flushed ova.

²Model [3b] vs. model [3a]: genetic effects of sire and dam are replaced by the genetic effect of the embryo.

fertility unaffected. According to the literature, genetic correlations between maternal and paternal effects for most fertility traits (e.g., the nonreturn rate measured at 90 d) are reported to be negative and assumed to be -0.05 for the official breeding value estimation in Germany (Pasman and Reinhardt, 1998). Studies revealing the physiological mechanisms for the phenomenon of slightly negative correlations between maternal and paternal effects for traits related to ET ought to be carried out in the future.

The genetic correlation between the direct effect of the recipient and the sire of embryos applying model

Figure 4. Genetic correlations in the synergistic model for the trait pregnancy after embryo transfer for the involved genetic groups (r_{mp} = genetic correlation between the donor cow and the sire of the embryo, r_{pr} = genetic correlation between the sire of the embryo and the recipient, r_{er} = genetic correlation between the embryo and the recipient, r_{mr} = genetic correlation between the donor cow and the recipient).

[3a] for pregnancy after ET was -0.32 and was -0.14 between the donor cow and the recipient (Figure 4). These results suggest that sires that should be preferred in selection for the improvement in conception ability of recipients were not necessarily best when considering the vitality of transferred embryos. These results are in agreement with the genetic correlation of -0.21 (Figure 4) between the embryo effect and the effect of the recipient, in which a simplification of the model was achieved due to the direct fit of the genetic effect of the embryo. The embryo genetic effects consist of half of the genes of the genetic mother and half of the genes of the genetic father plus the Mendelian sampling component; hence, these correlations were expected to be similar.

Following the results for pregnancy after ET, it can also be anticipated that fertility after artificial insemination is determined by a complexity of different components. This could be the vitality of the embryo itself combined with the metabolism and intrauterine environment of the cow, which are not necessarily positively correlated among each other. Results encourage the general attempt of Haussmann and Heinkel (1989) to develop synergistic models for fertility in dairy cattle. The genetic correlation between the effects of the donor cow and the sire of the embryo was 0.94, indicating no differences for embryo vitality whether genes are transmitted from the paternal or from the maternal side.

Genetic Correlations Between Different Traits

Because of the minor effect of the paternal component, only the maternal path was considered when estimating phenotypic and genetic correlations between different traits (Table 3). The ultimate breeding goal is to increase the number of TE, but correlations to other traits are important to find the most suitable breeding

Table 3. Estimates of genetic correlations (above diagonal; $\pm$ SE) and phenotypic correlations (below diagonal) for flushed ova (FO), transferable embryos (FE), degenerated embryos (DE), unfertilized oocytes (UO), PTE¹, and 305-d lactation yields for milk yield, fat percentage, protein percentage, and SCS for the maternal path (donor cow)

Trait	FO	TE	DE	UO	PTE	Milk, kg	Fat, %	Protein, %	SCS
FO									
TE	0.677	0.741 $\pm$ 0.080	0.898 $\pm$ 0.149	0.755 $\pm$ 0.092	-0.173 $\pm$ 0.100	-0.239 $\pm$ 0.087	-0.002 $\pm$ 0.055	0.003 $\pm$ 0.059	-0.347 $\pm$ 0.079
DE	0.310	0.160	0.722 $\pm$ 0.138	0.211 $\pm$ 0.142	0.562 $\pm$ 0.131	-0.272 $\pm$ 0.090	-0.004 $\pm$ 0.061	0.005 $\pm$ 0.060	-0.407 $\pm$ 0.088
UO	0.513	-0.001	-0.018	0.566 $\pm$ 0.168	-0.193 $\pm$ 0.158	-0.199 $\pm$ 0.095	0.211 $\pm$ 0.101	0.247 $\pm$ 0.104	0.181 $\pm$ 0.109
PTE	-0.072	0.684	-0.210	-0.663	-0.840 $\pm$ 0.111	-0.148 $\pm$ 0.091	-0.005 $\pm$ 0.099	0.001 $\pm$ 0.105	-0.125 $\pm$ 0.102
Milk, kg	-0.211	-0.308	-0.187	-0.111	-0.082	-0.036 $\pm$ 0.105	-0.003 $\pm$ 0.109	0.011 $\pm$ 0.110	-0.391 $\pm$ 0.112
Fat, %	-0.010	0.012	0.233	0.027	-0.091	-0.679	-0.566 $\pm$ 0.051	-0.440 $\pm$ 0.053	0.013 $\pm$ 0.075
Protein, %	-0.044	0.001	0.199	-0.082	-0.074	-0.550	0.771	0.810 $\pm$ 0.068	-0.192 $\pm$ 0.097
SCS	-0.591	-0.603	0.220	-0.008	-0.381	0.172	-0.344	-0.143	-0.130 $\pm$ 0.099

¹PTE = percentage of transferable embryos = no. of transferable embryos/no. of flushed ova.

strategy; for example, direct selection on TE or via indirect selection on FO as suggested by Bosselmann et al. (2005). The number of FO has a positive correlation with the numbers of TE ($r_g = 0.74$), despite the positive genetic correlation between FO and DE ($r_g = 0.89$), or between FO and UO ($r_g = 0.76$). The genetic correlation between FO and TE in the study by Preisinger et al. (1990) was 0.63. A higher number of FO resulted in increased embryonic losses due to infertility and degeneration, as indicated by the negative genetic correlation between FO and PTE ($r_g = -0.17$). Hence, breeding for increased FO also increases DE and UO, but cows selected for a high number of FO are still the best when regarding the number of TE. Indirect selection on FO will increase the selection response in TE by about 22% compared with direct selection on TE because of the higher heritabilities for FO found in the present study. Because of the pronounced genetic correlations associated among FO and TE, DE, and UO, the genetic correlations between TE and DE ($r_g = 0.52$) and TE and UO ($r_g = 0.21$) were also positive. A greater number of DE was genetically associated with a higher number of UO ($r_g = 0.57$). Phenotypic correlations between all these traits were in the same range as genetic correlations (Table 3).

As recently shown by Miglior et al. (2005), in most conventional dairy cattle breeding programs, selection is mainly focused on milk yield or production traits. Due to the high selection pressure on production, the success in other traits will largely depend on the genetic correlations between these traits and milk yield. Donor cows are highly preselected for milk yield and characterized by a high production level in milk yield greater than 9,962 kg per lactation (Table 1), but ET often failed. As shown in Figure 3, 22.6% of all flushes failed completely, resulting in zero TE. This could be because of the negative phenotypic and genetic correlations between milk yield and TE found in the present study. The genetic correlation between 305-d lactation milk yield and TE was -0.27 and the phenotypic correlation was -0.21. The phenotypic relationship between the production level and traits related to ET was analyzed in several previous studies (Wichmann, 1990; Manciaux et al., 2000). Manciaux et al. (2000) grouped donor cows according to their 305-d production level for milk yield. The average number of TE of cows producing more than 11,000 kg of milk was 4.9 compared with 7.5 TE of donor cows ranging between 9,000 and 11,000 kg of milk per year. Glatzel et al. (1999) focused their investigations on test day records when discussing phenotypic relationships between milk yield and TE. High levels of test-day milk yield were generally associated with fewer TE. An explanation could be that potential energy intake is insufficient to express further produc-

Table 4. Correlated selection response for flushed ova (FO), transferable embryos (TE), degenerated embryos (DE), unfertilized oocytes (UO), and the percentage of transferable embryos (PTE) when selecting on various production traits

Trait	FO	TE	DE	UO	PTE
Per 1,000-kg increase in milk yield	-0.94	-0.46	0.01	0.05	-0.04
Per 1% increase in fat percentage	-0.05	-0.18	0.23	-0.03	-0.01
Per 1% increase in protein percentage	0.13	0.01	0.07	0.02	0.09
Per 1-unit increase in SCS	-1.64	-0.80	0.05	-0.19	-0.33

¹PTE = percentage of transferable embryos = no. of transferable embryos/no. of flushed ova.

tion potential. Additional resources are drawn away from fitness-related traits such as fertility and health (Van der Waaij, 2004) and therefore, result in fewer TE.

Despite the optimization of ET when considering the amount of test-day milk yield, mainly to find the optimal point during a cow's lactation for flushing, breeding strategies can contribute to ensure more success of MOET breeding programs. Based on genetic parameters found in the present study, an index for potential bull dams including production traits and traits related to ET can be developed. Further studies in this field of research, including across-country studies, could contribute to the amount of data and ensure more reliable estimates for genetic variance and covariance components.

Genetic and phenotypic correlations among traits related to ET and the percentages of fat and protein were near zero (Table 3), except for DE. An increase of DE with increasing fat percentage was also found by Grunert and Berchthold (1999), who suggested that insufficient energy intake might be a contributing factor. Milk protein content, coupled with MUN levels, is related to energy balance and is a more accurate predictor of ET traits than protein content alone (Kafi and McGowan, 1997; Bosselmann et al., 2005). The genetic correlation between TE and SCS was -0.41 (Table 3), indicating that selection on improved udder health is associated with an increase in TE. A negative impact of SCS on nonreturn rates of cows was shown by Miller et al. (2001) and König et al. (2006). As indicated by the correlations found in the present study, fertility traits related to ET such as FO, TE, and PTE are also influenced by the status of udder health. Schrick et al. (2001) suggested an influence of clinical or subclinical mastitis on reproductive response by alterations in endocrine profiles and follicular development. Details of this physiological mechanism are given by Schrick et al. (2001) and Moore et al. (1991).

The correlated response in selection for different traits related to ET when selecting on different production traits is shown in Table 4. Correlated response using the genetic parameters in this study was calculated by $\Delta G_{ET,PT} = \frac{r_g \times \sigma_a(ET)}{\sigma_a(PT)}$, where the subscript *ET*

describes the various traits related to ET and the subscript *PT* indicates the investigated production traits. An increase in milk yield of 1,000 kg decreased the number of FO by 0.94 and decreased the number of TE by 0.46. As indicated by the low correlations in Table 3, even the unrealistically high increase of 1% for fat or protein percentage (Table 4) resulted in only minor changes in traits related to ET. The negative impact of SCS on TE, as expected when evaluating the genetic correlations in Table 3, was verified when focusing on the correlated selection response. An increase in SCS by 1 unit reduced the number of TE by 0.80 embryos. An index for potential bull dams after finishing their test at a central station, as developed by Kandzi (1988), should include nonproduction traits. An extension to traits related to ET as well as information about health such as SCS can contribute to greater success of existing MOET breeding schemes when following the suggestions given in the present study.

CONCLUSIONS

Potential accumulation of genetic gain is limited by biological constraints that, in combination with genetic parameters, determine the structure of breeding programs to be applied. Several theoretical investigations suggested the application of MOET breeding schemes, but their success often failed due to the limited number of offspring per donor. As shown in this study, analyzing traits related to ET includes a complexity of several genetic groups. However, results show the feasibility of estimating variance and covariance among these groups in a synergistic context applying GLMM for different specific distributions (normal, binomial, and Poisson) of traits. The present study revealed, based on the estimates for genetic parameters, additional potential to include traits related to ET in a combined breeding goal for potential bull dams. Due to the higher heritability found for FO compared with TE, correlated selection strategies should be applied when improving TE. The most complex model was used for the status of pregnancy of recipients involving genetic effects of recipients and both genetic parents of the embryos. Following the original idea of synergistic models (Willham,

1963), a fourth genetic component describing the vitality of the embryo itself could be included in statistical models. Nevertheless, the attempt presented in this study was the first to utilize synergistic models in the case of pregnancy after ET. Results revealed moderate antagonism between the direct effect of the recipient and the maternal and paternal effect contributing to an embryo's vitality. This finding was verified when replacing the maternal and paternal effect by the genetic effect of the embryo itself. The genetic impact of the recipient was nearly 6% of the total variance for pregnancy and was, therefore, more important for the ultimate success in ET than embryo vitality. However, the practical relevance of this result is limited, because intensive selection for fertility of recipients is currently not realistic.

Nevertheless, selecting for ET-related traits may be of increasing interest with a change to young sire breeding programs, as discussed in the context of introducing genomic selection in dairy cattle (Schaeffer, 2006), which will require short generation intervals and increased reproduction rates on all selection paths.

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