Genetic evaluation of twinning rate in Italian Holstein

F. Galluzzo[1,2], G. Visentin[2], J.B.C.H.M. van Kaam[1], R. Finocchiaro[1], M. Marusi[1] and M. Cassandro,[1,3]

[1] Associazione Nazionale Allevatori della razza Frisona, Bruna e Jersey Italiana (ANAFIBJ), Cremona, Italia
[2] Dipartimento di Scienze Mediche Veterinarie – Università di Bologna Alma Mater Studiorum, Bologna, Italia
[3] Dipartimento di Agronomia, Alimenti, Risorse Naturali, Animali e Ambiente – Università degli Studi di Padova, Legnaro, Italia
Corresponding author: ferdinandogalluzzo@anafibj.it

Abstract

Twinning in Holstein cattle is unfavorably linked to calving difficulties, abortions, milk production and reduced calf survival. The twinning rate in the Italian Holstein population is 2.2% and appears relatively stable over time; however, this figure does not account for early abortions and thus it is underestimated. This study aimed to establish a routine genetic evaluation of twinning rate in the aforementioned population. The phenotype of interest was the type of calving (0 = singleton; 1 = twins). The statistical model employed included, as random, herd-year of conception, permanent environmental effect and the cow's additive genetic effect. Fixed effects comprised year-season of conception, herd, synchronization protocol (classified into three categories: yes, partial or no), days in milk class and parity-age-year of conception. The dataset included 11,329,160 records after filtering, with age at calving restricted to 18-77 months and parity limited to maximum three. Only fixed effects levels comprising at least 100 observations were retained. The minimum number of contemporaries was set to 10. Data editing was loop-based to simultaneously meet all the described restrictions. Genetic parameters were estimated on a sample of 500 randomly selected herds using THRGIBBS1F90 software. Posterior mean of heritability for twinning rate was 1%. To validate the accuracy and stability of the predictions a genomic validation was conducted. Genomic validation yielded a dispersion of 0.94 and validation reliability of 0.18. This study has laid the foundation for the implementation of a routine genetic evaluation of twinning rate in the Italian Holstein breed.

Key words: twinning, dairy cattle, genomic selection, codominance, model validation, genetic parameters

Introduction

Twinning in cattle can result from either monozygotic or dizygotic embryos. Monozygotic (identical) twins arise when a single fertilized egg splits into two embryos, whereas dizygotic (fraternal) twins originate from the fertilization of two separate eggs by two different sperm cells. In the Holstein breed, the vast majority of twins are dizygotic, originating from multiple ovulations and separate fertilizations. The most likely cause of twinning is the codominance of multiple dominant follicles, which is often associated with low progesterone (P4) concentrations during the follicular waves (Martins et al, 2018). This hormonal environment alters the secretion patterns of follicle-stimulating hormone (FSH), promoting the simultaneous development of multiple dominant follicles.

Multiple pregnancies increase the risk of reproductive and metabolic disorders, stillbirth, freemartinism and early pregnancy loss: the estimated cost of a twin pregnancy ranges from $59 to $161 (Cabrera et al, 2021).

In the light of the above, the aim of this study was to implement twinning rate (TWI) into the routine genetic and genomic evaluation system for Italian Holsteins.

Materials and Methods

Data editing

Data after edits consisted of 12M records of calving events from 1987 onwards. Maximum parity order was set to three and the following ranges of age at calving within parity order were defined: 18-41 moths (parity one), 30-59 (parity two) and 42-77 (parity three). The days in milk (DIM) range at conception was 21-305 while the gestation length range was 240-315 days. The minimum number of contemporaries for herd-year of conception was 10. The minimum number of observations per level of fixed effect was 100. All the criteria were assured to be met with a loop-based approach. The classification of synchronization protocol application was derived from the weekly distribution of inseminations in each herd during each period, considering 2010 as lower cutoff year. When more than 75% of inseminations occurred on a specific day of the week, it was assumed that synchronization was applied to the entire herd. If the percentage ranged between 35% and 75%, partial application of the protocol was assumed. Below 35%, it was considered that no synchronization was applied. Farm sizes and time trends within farms were taken into account to enhance classification accuracy. Each group corresponds to different synchronization strategies and, consequently, to different expected effects on the phenotype. Pedigree was traced back to 3 generations.

Statistical model

A single trait repeatability linear animal model was used, with twinning (TWI) as dependent variable. Following a previous study on the Italian Holstein breed (Katende et al, 2025), it was possible to apply a linear model with the cow as the additive genetic effect. The choice of a linear model did not result in significant differences compared to the theoretically more appropriate threshold model. The direct effect, that of the sire, was found to be negligible and was therefore not included in the model.

The model was the following:

$$\mathrm{TWI}_{ijklmnop} = hy_i + S_j{*}Y_k + \mathrm{H}_\mathrm{l} + DIM_l + AGEC\_PAR_\mathrm{m}{*}Y_k + \mathrm{SYNC}_\mathrm{n} + a_o + pe_\mathrm{o} + e_{ijklmnop}$$

with $\mathrm{TWI}_{ijklmnop}$ as the pth binary (singleton/twin) phenotypic observation of twin calving. Fixed effects were $S_j{*}Y_k$ as the crossed effect of season j by year k of conception, H_l as herd of conception, DIM_l as the lth days in milk at conception class (10 classes of 30 days), $AGEC\_PAR_\mathrm{m}{*}Y_k$ as the mth age at calving by parity class (9 classes: 3 age at calving classes for every parity class) by year k. Random effects were hy_i as the ith contemporary group for herd-year of conception, a_o as the additive genetic effect of the oth cow, pe_o as the permanent environmental effect of the oth cow and $e_{ijklmnop}$ as the residual of observation p.

Variance components estimation, genetic and genomic evaluation, approximate genetic correlations

Variance components estimation was performed with the software THRGIBBS1F90 (Misztal et al, 2002) on a sample of 635,026 animals (500 herds). Convergence was assessed with R package BOA, Bayesian output analysis (Smith, 2007). Conventional estimated breeding values (EBVs) were estimated with MiX99 software (MiX99 development team, 2015). Genomic evaluation was performed with a SNPBLUP model using GS3 software (Legarra et al, 2011). For estimated deregressed proofs (EDPs), the method from Degano et al (2016) was applied. A conventional quality control was applied to SNP data. For the imputation process, PedImpute software was used (Nicolazzi et al, 2013). Approximate genetic correlations were calculated as Pearson correlation coefficients between genomic estimated breeding values (GEBVs) of 3,200 heifers born in 2025.

Conventional, genomic and phenotypic validation

Genomic validation was performed as described in Finocchiaro et al (2012) and Galluzzo et al (2022). Briefly, two datasets were used for EBVs estimation: one full (with records up to 2504 run) and one reduced (with a 4-years back cutoff date). For both sets of EBVs, EDPs were calculated and used as pseudo-phenotypes for SNP effects estimation. Bulls with daughters in the full datasets but without in the reduced one were selected as validation bulls. Finally, a linear regression with EDPs of validation bulls from the full run as dependent variable and their direct genomic values (DGVs) from the reduced run as the independent one was fitted.

Parameters considered were the dispersion coefficient and the reliability of the linear regression model (validation reliability). To assess the validity of predictions at phenotype level, a sample of 1,168 females with phenotypes in the full run but not in the reduced run was selected. Their DGVs from the reduced run were standardized, and the average phenotype was evaluated for each standardized category.

Results & Discussion

The average frequency of twinning across the entire dataset was 2.2%, in agreement with the literature (Kirkpatrick et al., 2025). The twinning rate in the Italian Holstein population is estimated at 2.2% It has remained fairly constant over time; however, this value is likely underestimated as it does not include early abortions. The posterior mean of heritability was estimated at 0.01 (posterior standard deviation: 0.001), which falls within the ranges reported in the literature (Kirkpatrick et al., 2025; Lett et al., 2018; Katende et al., 2025; Hüneke et al., 2025).

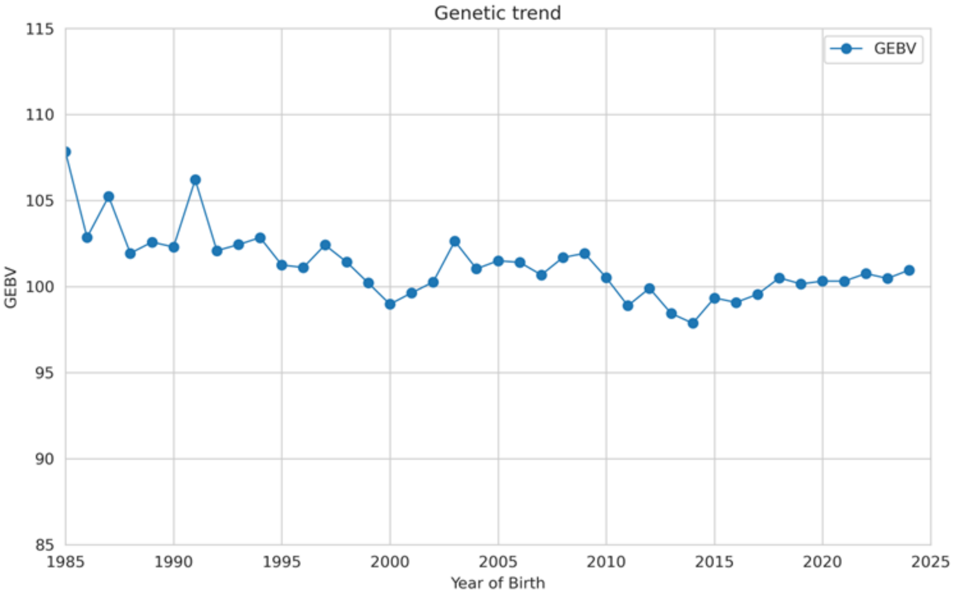

Figure 1. Bulls' genetic trend by birth year. GEBV=average GEBV.

This result confirms the potential for selection on this trait, despite the challenges posed by its low magnitude. GEBVs are expressed with a mean of 100 and a standard deviation of 5, with values above 100 referring to animals with lower genetic potential for twinning (and therefore considered favorable). The trend of GEBVs, reported in Figure 1, displayed a decline until the last 10 years, during which a reversal was observed, likely linked to improvements in traits related to female fertility. A similar trend was identified by Kirkpatrick (2025). Indeed, genetic selection for reducing twinning rate in dairy cattle may be desirable, provided it does not lead to undesirable correlated responses in other economically important traits. Twin births in dairy cattle are typically associated with negative outcomes, such as impaired reproductive performance and decreased calf survival.

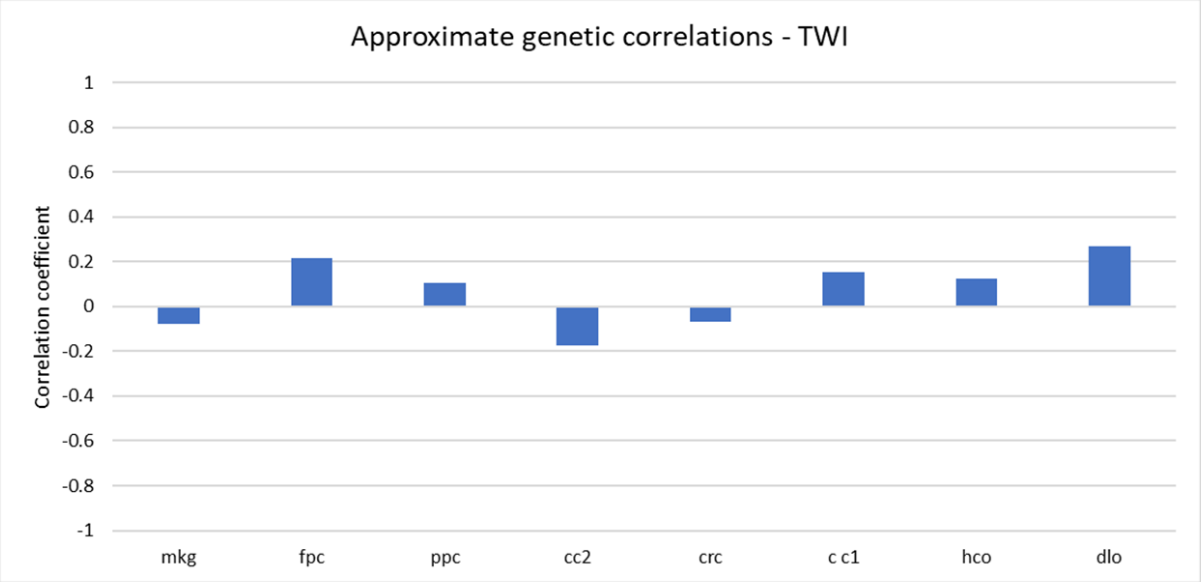

Figure 2. Approximate genetic correlations for TWI. Mkg=milk yield, fpc=fat percentage, ppc=protein percentage, cc2=interval first-last insemination cows, crc=cow recycling, cc1=conception rate cows, hco=conception rate heifers, dlo=direct longevity.

The approximate genetic correlations are depicted in Figure 2: for all traits related to fertility and milk quality, correlations were low but favorable. Regarding milk yield, a slight negative correlation was detected, probably due to a faster metabolism leading to more rapid degradation of P4.

The genomic validation resulted in a dispersion coefficient of 0.94, indicating a negligible overestimation of the DGV and confirming the accuracy and stability of both the statistical model and the genetic evaluation procedure. The validation reliability resulted 0.18.

Phenotypic validation, represented in Figure 3, confirms the accuracy of the predictions in relation to future phenotypes. Indeed, when considering only the groups with a sufficient number of observations to ensure a reliable mean (ranging from -2 to +1 standard deviations from the mean), we observe that higher genetic indices are associated with more favorable future phenotypes.

Conclusions

In conclusion, this study increased the knowledge about the genetic aspects of TWI in the Italian Holstein population and revealed the possibility to genetically improve the breed for this trait. Moreover, it confirmed the stability of the applied model and its ability to predict future phenotypes through genomic evaluation. Moreover, it confirmed the stability of the applied model and the ability of genomic evaluation to predict future phenotypes, thus providing solid decision-making support for the selection of breeding animals both at the AI center and farm level. A routine genetic evaluation of TWI will be soon implemented in Italy for the Italian Holstein breed.

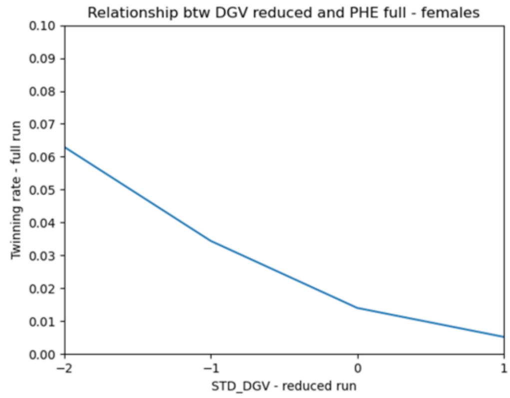

Figure 3. Average full-run phenotype (Twinning rate - full run) and standardized DGV (STD_DGV - reduced run) for females lacking phenotypes in the reduced run.

Acknowledgments

This study was supported by “Latteco2 project, sottomisura 10.2 of the PSRN-Biodiversity 2020–2023” (MIPAAF. D.M. no. 465907 del 24/09/2021, project unique code J12C21004080005).

The authors would like to thank Dr. Massimiliano Lanteri (DVM) and Dr. Stefano Allodi (DVM) for their valuable support and collaboration.

References

Cabrera VE, Fricke PM. 2021. Economics of Twin Pregnancies in Dairy Cattle. *Animals*.; 11(2):552. https://doi.org/10.3390/ani11020552

Degano, L., Jansen, G., Finocchiaro, R., Rossoni, A., Vicario, D. 2016. Hybrid One-Step Genomic Evaluation System for the Italian Simmental Breed. *Interbull Bulletin*. 50. 24-28. https://journal.interbull.org/index.php/ib/article/view/1624/1626

Finocchiaro, R., van Kaam, J.B.C.H.M. and Biffani, S. 2012. The Genomic Selection System in Italian Holstein. 2012. *63rd Annual Meeting of the European Federation of Animal Science*, Bratislava, Slovensko.

Galluzzo, F., van Kaam, J.B.C.H.M., Finocchiaro, R., Marusi, M., Tsuruta, S., and Cassandro, M. 2022. Estimation of milkability breeding values and variance components for Italian Holstein. *Journal of Dairy Science Communications*. 3. 180–184. https://doi.org/10.3168/jdsc.2021-0167

Hüneke L, Schmidtmann C, Alkhoder H, Liu Z, Segelke D, Rensing S, Heise J, Thaller G. 2025. Genetics of twin birth rate in German Holstein and options for breeding. *J Dairy Sci*. Jun;108(6):6161-6173. Epub 2025 Apr 16. PMID: 40250614. https://doi.org/10.3168/jds.2024-25972

Katende JS, Costa A, Santinello M, Galluzzo F, Marusi M, Finocchiaro R, Cassandro M, Penasa M. 2025. Genetics of twinning rate in Italian Holsteins. *J Dairy Sci*. May;108(5):5103-5113. Epub 2025 Mar 3. PMID: 40043761. https://doi.org/10.3168/jds.2024-25936

Kirkpatrick BW, Berry DP. 2025. A genomic analysis of twinning rate and its relationship with other reproductive traits in Holstein-Friesian cattle. *J Dairy Sci*. Feb;108(2):1686-1698. https://doi.org/10.3168/jds.2024-25236

Legarra, A., Ricardi, A., Filangi, O. 2011. GS3: Genomic Selection, Gibbs Sampling, Gauss-Seidel (and BayesCp).

Lett BM, Kirkpatrick BW. 2018. Short communication: Heritability of twinning rate in Holstein cattle. *J Dairy Sci*. May;101(5):4307-4311- Epub 2018 Feb 15. PMID: 29454683. https://doi.org/10.3168/jds.2017-13660

Martins JPN, Wang D, Mu N, Rossi GF, Martini AP, Martins VR, Pursley JR. 2018. Level of circulating concentrations of progesterone during ovulatory follicle development affects timing of pregnancy loss in lactating dairy cows. *J Dairy Sci*. Nov;101(11):10505-10525. Epub 2018 Sep 7. PMID: 30197145. https://doi.org/10.3168/jds.2018-14410

MiX99 Development Team. 2015. MiX99: A software package for solving large mixed model equations. https://www.luke.fi/en/services/mix99-solving-large-mixed-model-equations

Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D.H. 2002. BLUPF90 and related programs (BGF90). *7th World Congress on Genetics Applied to Livestock Production*, Montpellier, France. Google Scholar

Nicolazzi, E.L., Biffani, S. and Jansen, G. 2013. Short communication: Imputing genotypes using PedImpute fast algorithm combining pedigree and population information. 2013. *Journal of Dairy Science*. 96. 2649–2653. https://doi.org/10.3168/jds.2012-6062

Smith, B.J. 2007. boa: An R Package for MCMC Output Convergence Assessment and Posterior Inference. *Journal of Statistical Software.* 21. 1-37. https://doi.org/10.18637/jss.v021.i11