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Genetics of twinning rate in Italian Holsteins

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ABSTRACT

Twinning is undesirable in dairy cattle because it is associated with higher risks of abortion, calving difficulty, and metabolic and reproductive issues. Moreover, twin calves are lighter at birth and generally have a lower survival rate. As a result, twinning leads to substantial economic losses for the farmer. In the current study, genetic variance and h^2 of twinning rate (TR) were estimated as the first step to investigate the feasibility of reducing or at least curbing its increase in the Italian Holstein population through genetic strategies. Calving records (n = 1,625,859) were registered between 1992 and 2022 in 1,830 Holstein herds. A binomial logistic regression model was used to investigate the odds of TR across parities and calving seasons. The h2 and repeatability of TR were estimated using single-trait linear animal, linear sire, linear direct-maternal, threshold animal, threshold sire, and threshold direct-maternal models, which accounted for the fixed effect of parity, the random effects of herd-year-season of calving, permanent environment, and the residual and, depending on the model, the random animal, sire, or direct-maternal genetic effects. Moreover, a multiple-trait approach was adopted considering TR in different parities as different traits to estimate h2 within parity, as well as genetic and phenotypic correlations between parities. Similarly to the single-trait approach, linear and threshold animal, sire, and direct-maternal models were used. All models included the fixed effect of calving season, the random effects of herd-year of calving and the residual and, depending on the model, the random animal, sire, or direct-maternal genetic effects. The overall TR was 2.71% and 90% of the herds had TR from 0.00% to 4.49%. The greatest TR was observed after parity 2 (odds ratio ~5.20) compared with parity 1, and in summer (odds ratio = 1.32) compared with winter. The h^2 increased with parity, ranging from 0.005 (parity 1) to 0.029 (parity ≥4) with linear models, and 0.061 (parity 1) to 0.142 (parity 3) with threshold models. Regardless of the model used, the genetic correlations between parities ranged from moderate to strong (0.66–0.99). Also, genetic correlations were stronger between multiparous than between primiparous and multiparous cows. Pearson correlations between sires EBV for TR obtained from single-trait linear and threshold models were close to unity, hinting at a limited re-ranking of bulls. This result suggests that there is room to carry out genetic evaluation for TR with the linear animal model (which is easier to be implemented in routine genetic evaluation than the threshold model) and manipulate the occurrence of twins through genetic strategies in the Italian Holstein population in order to stabilize or reduce TR.

Key words: dairy cattle, twin, heritability, genetic correlation

INTRODUCTION

Cows generally produce a single calf per calving, although twin and rarely triplet are possible. The twinning rate (**TR**) ranges from 1.90% to 5.60% in dairy cattle, according to the breed or population investigated (Andreu-Vázquez et al., 2012; Kirkpatrick and Berry, 2025). In dual-purpose cattle, Atteneder (2007) observed a TR of 5.50% in Austrian Fleckvieh, and Caccin et al. (2025) reported multiple birth rate of 3.89% for Tyrol Grey and 5.80% for Pinzgauer. In beef cattle, the TR is generally lower than in dairy and dual-purpose cows, with values ranging from 0.57% for American Hereford (Johansson et al., 1974) to 5.03% for the Maine-Anjou breed (Manfredi et al., 1991).

Twinning is undesirable in dairy herds due to its direct and indirect negative impacts on dam fertility and health, as well as on calf health and survival. Indeed, twin pregnancy is associated with higher abortion risk (Day et al., 1995; Andreu-Vázquez et al., 2012), shorter gestation length, anomalous presentation of calves at

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-25. Nonstandard abbreviations are available in the Notes.

birth, and increased incidence of placental retention, dystocia (Echternkamp and Gregory, 1999; Echternkamp et al., 2007), and reproductive disorders such as metritis and endometriosis (Bell and Roberts, 2007). Moreover, metabolic diseases such as ketosis and displaced abomasum increase after a twin birth (Komisarek and Dorynek, 2002; Silva del Río et al., 2010). Cows that deliver twins are at a higher risk of experiencing longer days open and thus a longer calving interval than cows that deliver singletons (Hossein-Zadeh, 2010; Sawa et al., 2015), along with a shorter productive life (Bicalho et al., 2007; Andreu-Vázquez et al., 2012). On the calf side, twinning is associated with increased stillbirth (Silva del Río et al., 2007; Hossein-Zadeh et al., 2008), mortality, and lighter birthweight (Echternkamp and Gregory, 1999; Hossein-Zadeh, 2010). Consequently, twinning impacts both direct and indirect costs, and thus the profitability of the dairy herd. Economic losses associated with twin calving have been estimated to be approximately US$130 per twin calving in British herds (Eddy et al., 1991) and US$109 per twin calving in Dutch herds (Beerepoot et al., 1992). More recently, the economic cost of twinning in US dairy farms has been estimated to range from $97 to $225 per twin calving, thereby amounting to over $96 million per year (Mur-Novales et al., 2018).

The occurrence of twin calvings in dairy cattle is dependent on nongenetic factors such as the herd management and reproductive protocols adopted, the cow's age, and the ovulation or conception season (Kinsel et al., 1998; Johanson et al., 2001; Lett and Kirkpatrick, 2018). Several studies have reported the presence of genetic variability for twinning in cattle, although h^2 and repeatability (**t**) are low and dependent on the breed, population, pedigree depth, and statistical model (Hossein-Zadeh et al., 2009; Fitzgerald et al., 2014). Some authors have identified multiple genomic regions across several chromosomes that are associated with twinning in North American, Israeli, and Swiss Holstein populations (Cruickshank et al., 2004; Weller et al., 2008; Widmer et al., 2021). For example, Widmer et al. (2021) observed signals on bovine chromosome 11, with functional candidate genes *LHCGR* and *FSHR* encoding receptors for LH and FSH, which are known to influence the ovulation rate and female fertility. In a separate study, a SNP (Hapmap22923-BTA-129564) close to *ARHGAP8* and *TMEM200C* genes on chromosome 24 was linked to TR in the Italian Maremmana cattle breed (Moioli et al., 2017).

According to the Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana (ANAFIBJ; Cremona, Italy) there is growing concerns among farmers about the increase in TR in Italian Holsteins (ANAFIBJ, personal communication). Given the increased milk production obtained after decades of genetic pressure and targeted selection in Europe and North America (Britt et al., 2018) it is crucial to deepen sources of variation of TR, including genetic factors. This would help to assess the feasibility of implementing genetic selection strategies aimed at stabilizing or reducing the TR in dairy cattle. The impact of nongenetic and genetic factors has not yet been investigated in a broader perspective in Italian Holsteins, and thus the objectives of this study were to (1) explore the nongenetic factors affecting TR; (2) estimate the h^2 and t of TR; and (3) estimate the phenotypic ($\mathbf{r_p}$) and genetic correlations ($\mathbf{r_g}$) of twinning between parities in the Italian Holstein population.

MATERIALS AND METHODS

Data

A dataset of 1,630,363 calving records produced by 686,354 Italian Holstein cows from 1992 to 2022 in 1,830 herds was retrieved from the Associazione Italiana Allevatori (Rome, Italy). The variables in the dataset were the cow ID, sire ID, dam ID, herd, birth date, calving date, and parity. Moreover, information on the type of calving (0 = singleton, 1 = twins or triplets) and sex of the calf (or calves, in the case of multiple births) was available. Duplicate calving records and records with missing parents, missing calving type, and from cows of parity >10 were removed from the dataset. After editing, 1,625,859 calvings of 685,666 cows mated with 19,129 sires in 1,830 herds were available to investigate the genetic and nongenetic factors affecting TR. In the dataset, 52,554 calving events were present for each year on average, ranging from 30,571 in 2022 to 63,598 in 2011. The lowest number of calving records in 2022 was due to the partial availability of data from this year (January to September).

Statistical Analyses

Nongenetic Effects. Nongenetic factors affecting TR were investigated using the following logistic regression model with a binomial link function using the package lsme4 (Bates et al., 2015) in the R software (ver. 4.3.3; https://www.r-project.org/) to obtain the log odds of twinning:

$$Logit(\pi) = \mu + Parity_i + Season_j + Herd_k + Year_l,$$

where $Logit(\pi)$ is the log odds of twin calving; μ is the overall intercept of the model; $Parity_i$ is the fixed effect of the ith parity of the cow (i = 1, 2, 3, and ≥4, with the latter being a class of cows from parity 4–10); $Season_j$ is the fixed effect of the jth calving season (j = winter [December to February], spring [March to May], sum-

mer [June to August], and autumn [September to November]); $Herd_k$ is the random effect of the kth herd (k = 1 to 1,830); and $Year_l$ is the random effect of the lth calving year (l = 1992 to 2022). After back-transforming the coefficients to the response scale, LSM were computed in the R software with the lsmeans package (Lenth, 2016).

Estimation of Genetic Parameters. A pedigree of 2,162,974 animals spanning 9 generations was provided by ANAFIBJ. It included 146 unknown sire groups and 126 unknown dam groups. Marginal posterior distributions of (co)variance components were estimated using numerical integration via the Gibbs sampler, implemented in the GIBBSF90+ software from the BLUPF90 family of programs (Misztal et al., 2018). A single chain of 150,000 Gibbs samples was generated, with the burn-in period initially set to zero. In the post-Gibbs analysis performed through the POSTGIBBSF90 (Misztal et al., 2018), the first 50,000 samples were discarded as burn-in. The thinning interval of 100 cycles was then adopted to reduce the autocorrelation between the sampled values. The posterior mean was used as a point estimate of (co)variance components and related parameters, and posterior SD was used as uncertainty metrics of the parameters. The convergence of the models was assessed through the visual inspection of the time series plots of the estimates along with the descriptive statistics (i.e., mean, median, and mode) of the marginal posterior distribution. The chain length was increased when there was no convergence. At first, h^2 and t of TR were estimated through single-trait analysis across parities. Furthermore, a multiple-trait approach was used considering TR in different parities as different traits to estimate the within-parity h^2, and r_p and r_g between parities.

Single-Trait Analysis. For the univariate analysis, several approaches were adopted to estimate the variance components and genetic parameters of the TR. Specifically, twinning was analyzed using linear and threshold animal, sire, and direct-maternal models. The animal model was used to determine the individual animal genetic effects; the sire model was applied under the premise that the genetics of the bull can influence ovulation rate (Ron et al., 1990; Gregory et al., 1997); and the direct-maternal model was implemented to partition the additive genetic variance into the direct and maternal genetic effects. According to the approach, the phenotype under investigation was the calving type (singleton, or twin or triplet) in the linear models and the unobserved liability for twinning in the threshold models. All models accounted for the fixed effects of parity, as defined above, and the random effects of herd-year-season of calving, permanent environment, and the residual. In addition, depending on the model, the random animal, sire, or direct-maternal genetic effects were included.

The h^2 and t for linear and threshold animal models were computed as follows:

$$h^2 = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

$$t = \frac{\sigma^2_a + \sigma^2_{pe}}{\sigma^2_a + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

where σ^2_a, σ^2_{hys}, σ^2_{pe}, and σ^2_e are variances for the additive genetic animal, herd-year-season of calving, permanent environmental, and residual effects, respectively.

The h^2 and t for linear and threshold sire models were computed as follows:

$$h^2 = \frac{4\sigma^2_s}{\sigma^2_s + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

$$t = \frac{4\sigma^2_s + \sigma^2_{pe}}{\sigma^2_s + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

where σ^2_s is the sire genetic variance.

The maternal h^2 and t for linear and threshold direct-maternal models were computed as follows:

$$h^2 = \frac{\sigma^2_m}{\sigma^2_m + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

$$t = \frac{\sigma^2_m + \sigma^2_{pe}}{\sigma^2_m + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e},$$

where σ^2_m is the variance for maternal genetic effects. The direct genetic variance and the direct-maternal covariance estimated from the linear and threshold direct-maternal models were negligible. Therefore, direct heritability was assumed to be zero, and maternal h^2 and t were computed without including the direct genetic variance and direct-maternal covariance in the denominator of the formulas.

Variance components estimated through the single-trait analyses were used to obtain the EBV of TR for sires with reliability ≥0.50, and to explore the relationships between linear and threshold models through Pearson correlations.

Multitrait Analysis. A multiple-trait approach was adopted considering TR in different parities as different

traits to estimate h^2 within parity, and r_g and r_p between parities. Models used for the multiple-trait approach were those used for the single-trait analysis (i.e., linear and threshold animal, sire, and direct-maternal). All models included the fixed effect of calving season, the random effects of herd-year of calving, and the residual and, depending on the model, the random animal, sire, or direct-maternal genetic effects. Due to the large data size used in the present study, the multitrait analysis involving herd-year-season would have resulted in a very high number of levels for the contemporary group and thus a large computational time to estimate genetic parameters. Therefore, unlike in the single-trait approach, the herd-year was used instead of the herd-year-season as contemporary group in the multitrait analysis.

Depending on the model, h^2 was calculated by using the formulas applied in the single-trait approach, with the removal of the permanent environmental variance and the inclusion of herd-year instead of herd-year-season variance in the denominator. Because herd-year covariances and residual covariances across parities were assumed to be zero in all models, r_g and r_p were estimated as follows:

$$r_g = \frac{\sigma_g\left(y_1, y_2\right)}{\sqrt{\sigma^2_{g_1} \times \sigma^2_{g_2}}},$$

$$r_p = r_g \times \sqrt{h^2_1 \times h^2_2},$$

where $\sigma_g(y_1,y_2)$ is the genetic covariance between the twinning in different parities, $\sigma^2_{g_1}$ and $\sigma^2_{g_2}$ are genetic variances, and h^2_1 and h^2_2 are heritabilities estimated for parity y_1 and y_2.

RESULTS AND DISCUSSION

Twinning Rate

The overall TR in the Italian Holstein population was 2.71% (Table 1), which is higher than the TR (2.03%) observed in Polish Holsteins by Sawa et al. (2015) and slightly lower than the TR of 3.01% observed in more than 1 million Iranian Holsteins (Hossein-Zadeh et al., 2009). In a study involving more than 2 million calving events recorded on US Holsteins, Silva del Río et al. (2007) reported TR of 4.20%. Recently, a higher TR of 4.40% has been reported for US dairy cows by Schambow et al. (2021) based on more than 1.5 million calvings. However, the comparison of TR of the present study with TR of other studies is not straightforward due to factors such as differences in environmental conditions, climate, herd management (especially in terms of reproductive management, use of synchronization protocols, and feeding strategies), and breeding goals.

Cows that twinned once had a recurrence rate of 6.31%, indicating that few animals delivered twins more than once within their lifetime, thereby supporting the nonzero repeatability. This value is comparable to those reported in the literature: for instance, Hossein-Zadeh et al. (2008) observed a recurrence rate of 6.90% in Iranian Holsteins and Silva del Río et al. (2007) of 7.00% in US Holsteins. In general, the recurrence of twinning might be underestimated due to the higher prevalence of twin calvings in older cows that are likely to be culled before manifesting the phenotype in subsequent calving.

In this study, the sex ratio for singleton calvings was in line with the expectation (i.e., 48.1% of calvings produced a male and 51.9% a female). In the case of twins, 33.1% of calvings delivered female-female, 36.5% male-female, and 29.5% male-male. In the US Holstein population, Silva del Río et al. (2007) reported sex ratios of 53.3% males and 46.7% females for singleton calvings, and 26.3% female-female, 43.6% male-female, and 30.1% male-male for twin calvings. Hossein-Zadeh et al. (2008) reported slightly different sex ratios compared with the present study (i.e., 22.9% female-female, 48.9% male-female, and 28.2% male-male). In the last 20 years, the progressive increase in the use of sexed semen worldwide may have influenced the sex ratios of calves. In the present study, no information about the type of semen used was available; nevertheless, changes in the sex ratio

Table 1. Frequency of singleton births, twin births, and twinning rate in each calving season and parity, along with the overall twinning rate

Class	Singleton births	Twin births	Twinning rate (%)
Calving season			
Winter	435,421	10,811	2.42
Spring	313,291	7,988	2.49
Summer	394,268	13,440	3.30
Autumn	438,800	11,840	2.63
Parity			
1	521,514	4,288	0.82
2	422,955	13,621	3.12
3	279,435	11,646	4.00
≥4	357,876	14,524	3.90
Overall	1,581,780	44,079	2.71

observed in the last years in favor of females are likely due to the boosted use of the sexed semen in Italy. In fact, in our dataset, the percentage of females out of all calves born in each year was greater in recent than in past years: for example, 49.8% of calves in 2005 were females compared with 58.8% in 2022.

As depicted in Figure 1, 90% of the herds investigated in the present study had TR from 0.00% to 4.49%, and 10% had TR ≥4.50%. In US Holsteins, Kinsel et al. (1998) and Silva del Río et al. (2007) reported TR from 0.00% to 9.60%, and 0.3% to 12.0%, respectively. The large variation of TR across herds suggests that some farm-specific management factors, such as hormonal synchronization before timed artificial insemination, exert an influence on the TR. For instance, in the study of López-Gatius et al. (2023) the use of the G6-G synchronization protocol was associated with increased twin pregnancies in primiparous Spanish Holsteins. Synchronization protocols that boost the progesterone concentration during development of the preovulatory follicle generally decrease the double ovulation up to 3-fold with consequently lower twin pregnancies (Carvalho et al., 2019). Low hepatic progesterone levels may inhibit FSH and increase LH secretions from the pituitary gland. In this way, likelihood of the development of multiple dominant follicles increases and may result in double ovulation events (Harrison et al., 1990; Wiltbank et al., 2014). In our study, the effect of synchronization protocols on the TR was not explored due to unavailability of data. Semen choice is also a farmer-related factor; hence, preference for bulls with high genetic merit for a given trait or index may mask an indirect selection for double ovulation and twinning, due to pleiotropy or linkage.

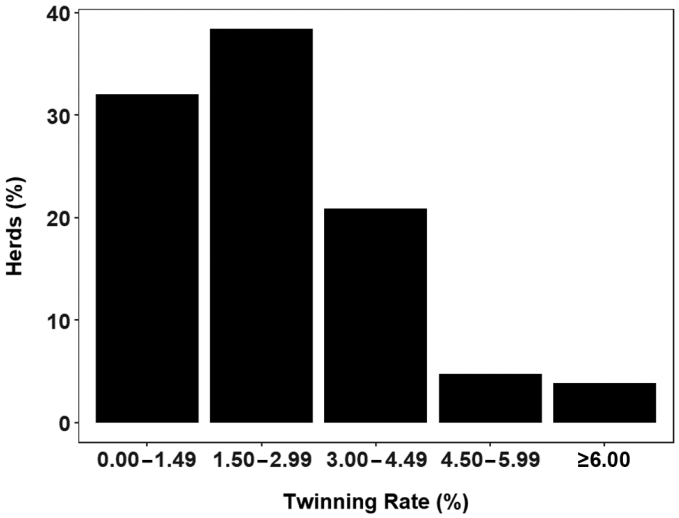

Figure 1. Distribution of herds (n = 1,830) across classes of twinning rate.

Nongenetic Effects

Calving Season. The probability of twin calving increased from 1.94% to 2.56% when moving from winter to summer. Indeed, the estimated odds of twinning in summer were 1.32 times the estimated odds of twinning in winter (Table 2). Other studies have observed a similar seasonal trend of twin calving in Holsteins. For instance, Sawa et al. (2015) observed TR of 2.41% in cows that calved in summer (i.e., mated in late autumn or winter), which was higher than the TR of cows that calved in winter (1.79%; i.e., mated in late spring or summer). Schambow et al. (2021) reported peaks of twinning for cows calving in June and July (5.40%) and the lowest TR (3.68%) for cows calving in March. This implies that conceptions occurring in late autumn or early winter are more likely to result in twin calves compared with other periods of the year. Seasonal factors, namely photoperiod, temperature, and heat load, play a crucial role on the reproductive sphere and hormonal regulation. In a study on primiparous Spanish Holsteins, López-Gatius et al. (2023) observed that the odds of conception of twins were 3.5 times greater during negative photoperiods (i.e., when daylight hours decrease) compared with months with an increasing photoperiod (January to May in the Northern hemisphere).

Parity. The probability of twin calving increased from 0.68% to 2.67% when moving from first- to second-parity cows, and to ~3.45% when moving to third- and fourth-or-greater-parity cows. Indeed, the estimated odds of twinning for second-, and for third- and fourth-or-greater-parity cows were 3.96 times and ~5.20 times the estimated odds of twinning for primiparous cows, respectively (Table 2). This pattern has been observed in other populations, such as North American Holsteins, various Norwegian breeds, and Israeli Holsteins (Karlsen et al., 2000; Bicalho et al., 2007; Weller et al., 2008), and can be partly explained by factors related to the physiology of reproduction: indeed, multiparous cows are known to generally experience multiple ovulations more frequently than primiparous cows (Macmillan et al., 2018). Supporting this, Kusaka et al. (2017) observed that multiparous cows had 7.90 times greater odds of double ovulations compared with primiparous. More than the r_p (0.06–0.26), the moderate to strong r_g (0.66–0.90) between twinning and multiple ovulations supports this dependency (Van Vleck et al., 1991; Fitzgerald et al., 2014).

Heritability and Repeatability

The estimates of variance components, h^2, and t for TR from the single-trait models are summarized in Table 3. The h^2 obtained from threshold models were higher

Table 2. Least squares means, odds ratios, and 95% CI of twinning for the fixed effects of calving season and parity[1]

Effect	LSM	95% CI	Odds ratio	95% CI
Calving season				
Winter	0.0194	0.0187–0.0202	Reference class	—
Spring	0.0222	0.0214–0.0231	1.15	1.11–1.18
Summer	0.0256	0.0247–0.0266	1.32	1.29–1.36
Autumn	0.0201	0.0194–0.0209	1.04	1.01–1.06
Parity				
1	0.0068	0.0066–0.0072	Reference class	—
2	0.0267	0.0257–0.0277	3.96	3.83–4.10
3	0.0346	0.0334–0.0359	5.18	5.00–5.37
≥4	0.0344	0.0332–0.0357	5.15	4.97–5.33

[1]Standard errors of LSM and odds ratios were <0.001 and ≤0.018, respectively.

than those from the linear approach. Regarding the linear models, maternal h^2 (0.019) was slightly greater than h^2 (0.017) from the linear animal model. Maternal h^2 resembled the estimate reported by Atteneder (2007) for Austrian Fleckvieh cows. Heritabilities from the linear sire (0.012) and linear animal model (0.017; Table 3) were comparable to the finding of Moioli et al. (2017), who estimated h^2 of 0.014 using linear animal model in the Italian Maremmana local beef cattle breed. For sire model analysis, Lett and Kirkpatrick (2018) reported slightly higher h^2 in US Holsteins compared with the present study (i.e., 0.0192 and 0.142 from linear sire and threshold sire models, respectively). In fact, in our study, the threshold sire model provided h^2 of 0.081 (Table 3). In general, the h^2 from the present study (from 0.012 to 0.113) falls within the range of other Holstein populations. The t was very low, with estimates from 0.019 to 0.049 for linear models and 0.068 to 0.173 for threshold models. This somewhat supports the low recurrence rate observed in this study. To the authors' knowledge, only a few studies have reported t of twinning in cattle. Lett and Kirkpatrick (2018) estimated t of 0.044 and 0.231 with linear and threshold sire models, respectively, and Caccin et al. (2025) estimated t of 0.05 in Austrian dual-purpose cows.

Figure 2 depicts the relationships between EBV for sires with reliability ≥0.50 obtained from linear and threshold single-trait models. Pearson correlations between the linear and threshold animal, linear and threshold sire, and linear and threshold direct-maternal models were 0.95, 0.99, and 0.96, respectively, which suggests strong accordance between the models (Figure 2). These results are of great importance in the framework of potential future genetic evaluation for TR. Indeed, even if the use of threshold models is advised to handle categorical traits like TR, our findings portend a very limited re-ranking when using a linear instead of threshold approach. This has important practical implications because in the rou-

Table 3. Posterior mean for herd-year-season of calving $\left(\sigma^2_{hys}\right)$ permanent environmental $\left(\sigma^2_{pe}\right)$, additive genetic animal $\left(\sigma^2_{a}\right)$, sire $\left(\sigma^2_{s}\right)$, maternal $\left(\sigma^2_{m}\right)$, residual $\left(\sigma^2_{e}\right)$, and phenotypic variance $\left(\sigma^2_{p}\right)$, along with h^2 and repeatability (t) for twinning rate across single-trait models; the posterior SD of h^2 and t is reported in parentheses

	Model					
Item	Linear animal	Linear sire[1]	Linear direct-maternal[2]	Threshold animal	Threshold sire[1]	Threshold direct-maternal[2]
σ^2_{hys}	0.00025	0.00026	0.00024	0.06502	0.06602	0.06910
σ^2_{pe}	0.00003	0.00068	0.00078	0.00572	0.00108	0.07698
σ^2_{a}	0.00046	—	—	0.07215	—	—
σ^2_{s}	—	0.00008	—	—	0.02211	—
σ^2_{m}	—	—	0.00051			0.14614
σ^2_{e}	0.02557	0.02517	0.02493	1	1	1
σ^2_{p}	0.02631	0.02619	0.02646	1.14289	1.08921	1.2922
h^2	0.017 (0.001)	0.012 (0.001)	0.019 (0.001)	0.063 (0.001)	0.081 (0.005)	0.113 (0.006)
t	0.019 (0.001)	0.038 (0.003)	0.049 (0.002)	0.068 (0.001)	0.082 (0.005)	0.173 (0.005)

[1]The h^2 for linear and threshold sire models was calculated as $h^2 = 4\sigma^2_s \Big/ \left(\sigma^2_s + \sigma^2_{hys} + \sigma^2_{pe} + \sigma^2_e\right)$.
[2]The direct genetic variance and the covariance between direct and maternal genetic effects was negligible.

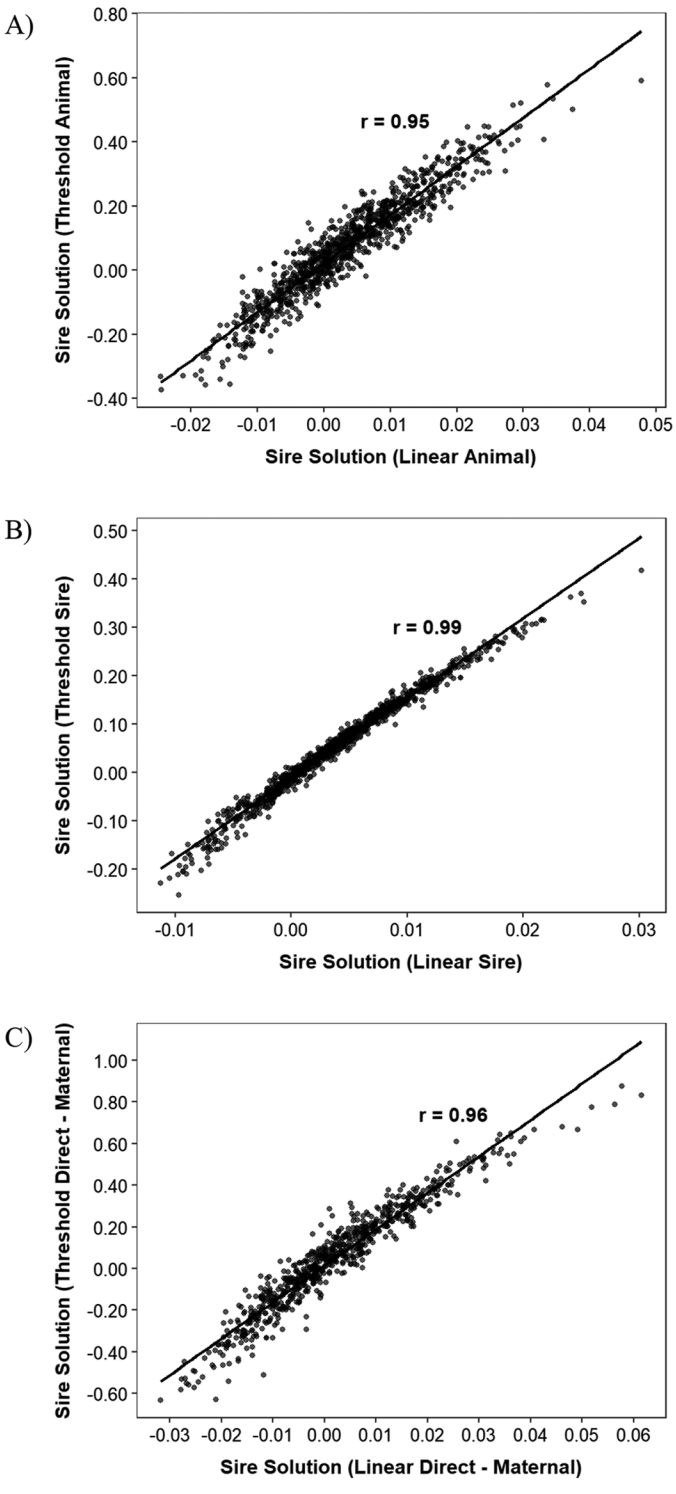

Figure 2. Distribution of sire solutions for twinning rate with reliability ≥0.50 from the single-trait linear versus the threshold (A) animal, (B) sire, and (C) direct-maternal models.

tine genetic evaluation linear models are preferred as easier to implement and manage compared with threshold models.

Within-Parity Genetic Parameters

Heritability. Results from the multitrait approach indicated that h^2 increased with parity, regardless of if a linear or a threshold approach was used (Table 4). For example, h^2 estimated from linear animal model increased from 0.005 to 0.018, and h^2 estimated from threshold animal model increased from 0.061 to 0.091 when moving from parity 1 to parity ≥4. Notably, the h^2 calculated using the linear models were similar (Table 4). Although the estimates are low, other studies observed that h^2 increases with parity. For instance, by using linear animal models Karlsen et al. (2000) estimated h^2 of 0.008 and 0.028 in first- and second-parity Norwegian cattle, and Weller et al. (2008) reported estimates from 0.01 in first-parity to 0.03 in fifth-parity Israeli Holsteins. Moreover, maternal h^2 from the threshold maternal model (0.082–0.140; Table 4) aligned with the findings of Masuda et al. (2015), who reported estimates between 0.11 and 0.16 in first-, second-, and third-parity Japanese Holsteins. The h^2 from linear models are similar to the estimates generally observed for mastitis, ketosis, interval from first to last insemination, and conception rate, which are currently under routine genetic evaluation in the Italian Holstein cattle (Ferrari et al., 2023; ANAFIBJ, 2024), and to health traits such as cystic ovaries, metritis, ketosis, retained placenta, and displaced abomasum, which exhibited h^2 from 0.01 to 0.04 in the Canadian Holsteins (Oliveira et al., 2021). In the Austrian Fleckvieh population, h^2 for ovarian cysts and retained placenta estimated with linear animal model in the first 150 DIM were 0.037 and 0.005, respectively (Costa et al., 2019).

Correlations. The r_p were weak (0.054–0.138) with threshold models and even weaker (0.007–0.027) with the linear approach (Table 5). Similarly, Hossein-Zadeh et al. (2009) reported r_p between twinning in different parities that were weak under both threshold (0.01–0.07) and linear models (0.01–0.05). The r_g were much stronger than the r_p (Table 5) and close to unity between higher parities. The r_g between parity 1 and ≥4, for example, ranged from 0.69 to 0.95, between parity 2 and ≥4 from 0.82 to 0.98, and between parity 3 and ≥4 from 0.88 to 0.98. Other studies have observed stronger r_g between twinning of multiparous cows (e.g., parity 3 and 4) than between parities 1 and 2. Weller et al. (2008) reported r_g of 0.805, 0.793, and 0.784 between parities 1 and 2, 1 and 3, and 1 and 4, respectively, and from 0.876 to 0.977 between parity 2 and later parities, and Masuda et al. (2015) estimated r_g of 0.92, 0.94, and 0.98 for parities 1 and 2, 1 and 3, and 2 and 3, respectively. Apart from the magnitude of correlations, it is important to emphasize that in the modern Holstein populations, for which the average productive life does not on average exceed 2.5 to 3 lactations, the twin calving recorded in parities up

Table 4. Posterior mean for herd-year of calving $\left(\sigma^2_{hy}\right)$, additive genetic animal $\left(\sigma^2_a\right)$, sire $\left(\sigma^2_s\right)$, maternal $\left(\sigma^2_m\right)$, residual $\left(\sigma^2_e\right)$, and phenotypic variance $\left(\sigma^2_p\right)$, and h^2 for twinning rate from multitrait models, where twinning rate in different parity classes was treated as different traits; the posterior SD of h^2 is reported in parentheses

Model	Parity 1	Parity 2	Parity 3	Parity ≥4
Linear animal				
σ^2_{hy}	0.00007	0.00029	0.00039	0.00037
σ^2_a	0.00004	0.00035	0.00077	0.00067
σ^2_e	0.00797	0.02966	0.03748	0.03659
σ^2_p	0.00808	0.03030	0.03864	0.03763
h^2	0.005 (0.0003)	0.012 (0.001)	0.020 (0.002)	0.018 (0.001)
Linear sire[1]				
σ^2_{hy}	0.00008	0.00029	0.00039	0.00038
σ^2_s	0.00001	0.00012	0.00023	0.00023
σ^2_e	0.00798	0.02982	0.03785	0.03688
σ^2_p	0.00807	0.03023	0.03847	0.03749
h^2	0.005 (0.001)	0.016 (0.001)	0.024 (0.001)	0.025 (0.001)
Linear direct-maternal[2]				
σ^2_{hy}	0.00007	0.00027	0.00035	0.00034
σ^2_m	0.00004	0.00061	0.00105	0.00107
σ^2_e	0.00797	0.02933	0.03697	0.03606
σ^2_p	0.00808	0.03021	0.03837	0.03747
h^2	0.005 (0.001)	0.020 (0.001)	0.027 (0.001)	0.029 (0.001)
Threshold animal				
σ^2_{hy}	0.0956	0.0589	0.0560	0.0569
σ^2_a	0.0708	0.0946	0.0989	0.1063
σ^2_e	1	1	1	1
σ^2_p	1.1664	1.1535	1.1549	1.1632
h^2	0.061 (0.004)	0.082 (0.003)	0.086 (0.005)	0.091 (0.004)
Threshold sire[1]				
σ^2_{hy}	0.0968	0.0566	0.0563	0.0542
σ^2_s	0.0227	0.0258	0.0281	0.0307
σ^2_e	1	1	1	1
σ^2_p	1.1195	1.0824	1.0844	1.0849
h^2	0.081 (0.009)	0.095 (0.008)	0.104 (0.008)	0.113 (0.005)
Threshold direct-maternal[2]				
σ^2_{hy}	0.0979	0.0567	0.0556	0.0538
σ^2_m	0.0981	0.1244	0.1753	0.1710
σ^2_e	1	1	1	1
σ^2_p	1.1960	1.1811	1.2309	1.2248
h^2	0.082 (0.003)	0.105 (0.004)	0.142 (0.004)	0.140 (0.005)

[1]Heritability for linear and threshold sire models was calculated as $h^2 = 4\sigma^2_s \Big/ \left(\sigma^2_s + \sigma^2_{hy} + \sigma^2_e\right)$.
[2]The direct genetic variance and the covariance between direct and maternal genetic effects was negligible.

to the third is expected to be of most interest for genetic selection purposes.

CONCLUSIONS

This study revealed that genetic variation exists for TR in the Italian Holstein population with h^2 up to 0.028 with linear and 0.142 with threshold models. The nonzero additive genetic variance indicates that the use of genetic selection is potentially feasible to alter the TR in this population in order to reduce or stabilize the incidence of twins with benefits for both the cows' and calves' performance, survival, and health. Genetic evaluation of sires for TR highlighted a strong accordance between the linear and threshold approach, as negligible changes in the rank of sires were observed. However,

Table 5. Posterior mean (posterior SD in parentheses) for genetic (r_g) and phenotypic correlations (r_p) of twinning rate between parities across various models

Model	Parity pair	r_g	r_p
Linear animal	1 vs. 2	0.86 (0.017)	0.007 (0.0004)
	1 vs. 3	0.87 (0.015)	0.008 (0.0007)
	1 vs. ≥4	0.91 (0.010)	0.009 (0.001)
	2 vs. 3	0.95 (0.006)	0.014 (0.001)
	2 vs. ≥4	0.97 (0.005)	0.014 (0.001)
	3 vs. ≥4	0.95 (0.006)	0.018 (0.001)
Threshold animal	1 vs. 2	0.78 (0.014)	0.055 (0.002)
	1 vs. 3	0.74 (0.016)	0.054 (0.002)
	1 vs. ≥4	0.76 (0.017)	0.057 (0.002)
	2 vs. 3	0.90 (0.005)	0.076 (0.004)
	2 vs. ≥4	0.91 (0.005)	0.079 (0.004)
	3 vs. ≥4	0.94 (0.005)	0.083 (0.004)
Linear sire	1 vs. 2	0.76 (0.053)	0.007 (0.001)
	1 vs. 3	0.75 (0.056)	0.008 (0.001)
	1 vs. ≥4	0.70 (0.048)	0.008 (0.001)
	2 vs. 3	0.92 (0.016)	0.018 (0.001)
	2 vs. ≥4	0.85 (0.046)	0.017 (0.001)
	3 vs. ≥4	0.88 (0.038)	0.022 (0.001)
Threshold sire	1 vs. 2	0.68 (0.064)	0.060 (0.007)
	1 vs. 3	0.66 (0.068)	0.061 (0.007)
	1 vs. ≥4	0.69 (0.081)	0.066 (0.008)
	2 vs. 3	0.90 (0.029)	0.089 (0.006)
	2 vs. ≥4	0.82 (0.076)	0.085 (0.007)
	3 vs. ≥4	0.88 (0.079)	0.095 (0.008)
Linear direct-maternal	1 vs. 2	0.96 (0.002)	0.009 (0.002)
	1 vs. 3	0.94 (0.002)	0.011 (0.002)
	1 vs. ≥4	0.92 (0.005)	0.011 (0.005)
	2 vs. 3	0.98 (0.001)	0.023 (0.001)
	2 vs. ≥4	0.96 (0.003)	0.023 (0.003)
	3 vs. ≥4	0.97 (0.002)	0.027 (0.002)
Threshold linear-maternal	1 vs. 2	0.97 (0.004)	0.090 (0.004)
	1 vs. 3	0.96 (0.004)	0.104 (0.004)
	1 vs. ≥4	0.95 (0.002)	0.101 (0.004)
	2 vs. 3	0.99 (0.004)	0.121 (0.004)
	2 vs. ≥4	0.98 (0.001)	0.118 (0.004)
	3 vs. ≥4	0.98 (0.001)	0.138 (0.005)

the response to genetic selection is expected to be slow due to the low h^2, and before proposing any stabilization strategies, genetic relationships between TR and both production and functional traits under official genetic evaluation should be explored. Furthermore, exploring genomic regions affecting the twinning occurrence is advisable.

NOTES

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Nonstandard abbreviations used: ANAFIBJ = Associazione Nazionale Allevatori della Razza Frisona, Bruna e Jersey Italiana; r_g = genetic correlation; r_p = phenotypic correlation; t = repeatability; TR = twinning rate.

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