ABSTRACT

Achieving an acceptable level of fertility in herds is difficult for many dairy producers because identifying cows in estrus has become challenging owing to poor estrus expression, increased herd size, and lack of time and skilled labor for estrus detection. As a result, synchronization of estrus is often used to manage reproduction. The aims of this study were (1) to identify artificial inseminations (AI) that were performed following synchronization and (2) to assess the effect of synchronization on genetic parameters and evaluation of fertility traits. This study used breeding data collected between 1995 and 2021 from over 4,600 Australian dairy herds that had at least 30 matings per year. Because breeding methods were not reported, the recording pattern of breeding dates showing a large proportion of the total AI being recorded on a single date of the year served as an indicator of synchronization. First, the proportion of AI recorded on each day of the year was calculated for each herd-year. Subsequently, synchronization was defined when a herd with, for instance, only 30 matings in a year, had at least 0.20 or more AI on the same day. As the number of breedings in a herd-year increased, the threshold for classifying AI was continuously reduced from 0.20 to as low as 0.03 under the assumption that mating of many cows on a single date becomes increasingly difficult without synchronization. From the current data, we deduced that 0.11 of all AI were possibly performed following synchronization (i.e., timed AI, TAI). The proportion of AI classified as TAI increased over time and with herd size. Although the deviation from equal numbers of mating on 7 d of the week was not used for classifying AI, 0.44 of AI being categorized as TAI were performed on just 2 d of the week. When data classified as TAI were used for estimating genetic parameters and breeding values, the interval between calving and first service (CFS) was found to be the most affected trait. The phenotypic and additive genetic variance and heritability, as well as variability and reliability of estimated breeding values of bulls and cows for CFS were lower for TAI than for AI performed following detected estrus (i.e., estrus-detected AI, EAI). For calving interval, first service nonreturn rate (FNRR), and successful calving rate to first service, genetic correlations between the same trait measured in TAI and EAI were close to 1, in contrast to 0.55 for CFS. The lower genetic variances and heritabilities for FNRR and calving interval in TAI than in EAI suggests that synchronization reduces the genetic variability of fertility. In conclusion, TAI makes CFS an ineffective measure of fertility. One approach to minimize this effect on genetic evaluations is to identify TAI (using the method described for example) and then set the CFS of these cows as missing records when running multitrait genetic evaluations of fertility traits that include CFS. In the long term, the most practical and accurate way to reduce the effect of synchronization on genetic evaluations is to record TAI along with mating data. 

Key words: reproductive synchronization, heritability, genetic parameters, evaluation

INTRODUCTION

Successful female fertility in dairy cattle depends on the ability of cows to express visible estrus following calving and to establish and maintain pregnancy after breeding in a timely manner. However, identifying cows that show estrus has become more challenging in recent years owing to an increase in the number of noncycling cows and an increase in the number of cows with poor or irregular estrus. In addition, management factors such as increased herd size and a shortage of both trained staff and time for monitoring cows in estrus (Lynch et al., 2021) have reduced the effectiveness of AI in dairy herds. The decline in estrus expression is in part a consequence of intense selection for milk yield over the past 30 to 50 years (e.g., Dobson et al.,
To reduce the need for identifying cows in estrus, reproductive intervention strategies that include synchronization of follicular growth, regression of the corpus luteum, ovulation, and insemination have become part of the management of dairy herds (Ribeiro et al., 2012). Since the late 1990s, several reproductive management protocols using a variety of hormones have been developed to improve fertility in dairy cattle (Lucy, 2019). However, the use of precision livestock farming for facilitating estrus detection and recording (Mottram, 2016) can be more acceptable to the general public than the use of hormones.

The extent of estrus synchronization has been increasing in recent years, although it still varies among dairy herds around the world (Caraviello et al., 2006; Higgins et al., 2013; Denis-Robichaud et al., 2016; Van Doormaal, 2018). According to a recent estimate, over 70% of dairy cows in the United States are treated with hormones for synchronization (Brotzman et al., 2015).

In Australia, the number of herds that use synchronization protocols has been growing in recent years. A survey conducted by Dairy Australia showed that the use reproductive intervention varies from 29% in small herds to 72% in large herds (S. Bullen, Dairy Australia, Melbourne, Australia, personal communication). The use of reproductive hormones to induce estrus means that traits such as the interval from calving to first service (CFS), or submission rate, may have a diminished value as fertility measures (e.g., Lucy, 2019). Currently, the genetic evaluation model for female fertility in Australia includes CFS as 1 of 5 traits (Haile-Mariam and Pryce, 2012). In seasonal calving systems, where mating as early as possible is desirable for achieving a tight calving pattern, this trait is assumed to be an important component of fertility (Grosshans et al., 1997; Haile-Mariam et al., 2004). The CFS is also included in multitrait genetic evaluation models in several Interbull member countries (Fleming et al., 2019; Interbull, 2022).

When data from cows that undergo synchronization are used for estimating genetic parameters (Goodling et al., 2005) and genetic evaluations (Lynch et al., 2021; Oliveira Junior et al., 2021), CFS would be the trait most affected. However, quantifying the levels of bias on genetic parameters and evaluations has been difficult (Goodling et al., 2005). First, accurately identifying synchronized (i.e., timed AI, TAI) and estrus-detected AI (EAI) is a challenge because the breeding method is generally not recorded (e.g., Lynch et al., 2021). The second challenge is the ability to quantify the effect of TAI on CFS and on other fertility traits related to the outcome of the inseminations (Goodling et al., 2005; Lynch et al., 2021; Oliveira Junior et al., 2021).

In this study, we first developed a simple heuristic method for classifying AI within a herd into 2 categories based on the proportion of AI recorded on a single date of the year (relative to the total number of recorded matings). This method relies on the recorded pattern of breedings and can indirectly indicate the use of estrus synchronization as well as some anomalies in the recording of breeding dates. After the breedings were classified, we assessed the effect of TAI on genetic parameters and genetic evaluation for CFS, calving interval, and success of insemination using nonreturn rate and successful calving rate to the first AI. To summarize, the objectives this study were (1) to quantify the extent of use of synchronization and TAI practices and (2) to assess the effect of TAI on genetic parameters and evaluation of fertility traits through a comparison with EAI.

MATERIALS AND METHODS

Mating data collected between January 1995 and April 2021 were extracted from the DataGene Ltd. (Bundoora, Victoria, Australia) database. Herds that had at least 30 AI in a year were selected. Between 1995 and 2021, a total of 11.8 million matings recorded in 4,612 herds were used. The mating data were merged with calving and lactation data of the cows that calved between 1994 and 2021, yielding 5.25 million records of 2.44 million cows with calving and the first service (FS) data. For this study, data that were already collected and stored for genetic evaluation were used. The data analysis did not require approval of Animal Care and Use Guidelines.

Identification of TAI

A heuristic approach was used to identify AI that most probably involved synchronization, using the proportion of AI on each date of the year as an indicator. The approach was developed following consultation with experts that work closely with the Australian dairy industry. Our classifications were also compared with 2 other methods previously developed to classify matings as TAI or EAI; these methods were based on the expectation that the number of AI on each day of the week should be equal in the absence of synchronization (Miller et al., 2007; Batista et al., 2016).

To identify TAI, each herd’s total number of matings in a year was first determined, and then the proportion of AI on each day of the year was calculated. If the proportion of matings on a particular date was higher than expected, we assumed that synchronization occurred. For example, for a herd with 30 mat-
ings in a year, if 0.20 (i.e., 6) were mated on the same date, then this was arbitrarily assumed to be due to synchronization. For herds with a small number of matings, 0.20 of the total matings on a single date was used as a threshold. For larger herds, the threshold was decreased iteratively to as low as 0.03, which was the case when the total number matings recorded in the herd-year was greater than 4,000. Figure 1 illustrates the relationship between the total number of matings in a herd-year and the proportion of AI performed in a single date of the year, which was used as the threshold for classification of AI. The justification for reducing the threshold with an increasing number of breedings was that the ability to perform many AI on a single date would become increasingly more challenging without synchronization. In addition, larger herds were more likely to perform synchronization than smaller herds. Furthermore, large herds must mate more cows to reach the same threshold because they have more cows to mate. For example, a herd with 4,000 AI in a year would have to mate 120 cows (i.e., a threshold of 0.03) in a single day to be classified as TAI. Although a threshold of 0.20 for herds with small number of matings was arbitrarily made, the proportion of matings classified as TAI relative to the total number was not very sensitive to the starting threshold. Indeed, we obtained similar results of classification with a threshold of 0.15 or even 0.10. In addition, matings from herd-years that did not report any mating on Sunday or Saturday were classified as TAI after consultation with practitioners (A. Daniel, Bull Nu-Genes, Terang, Victoria, Australia, personal communication). The rationale was that herds that do not practice synchronization are more likely to have mating on all days of the week, including weekends.

We also used a $\chi^2$ test-based method (Miller et al., 2007), which classifies all breedings in a herd-year as TAI if the observed number of breedings on each day of the week differs from the expectation. This method assumes that any significant deviation from an equal number of breedings on each day of the week is possibly due to synchronization. For classifying AI, we tested a minimum mean $\chi^2$ adjusted for herd size. Because preliminary results from this method showed the number of matings categorized as TAI was less than that classified by our proposed method (proportion of AI), we assumed a less stringent criterion of a minimum mean $\chi^2$ value of 45 (possible, probable, and synchronized) and 84 (probable and synchronized) to classify herd-years as TAI (Miller et al., 2007).

Figure 1. Relationship between the total number of AI in the herd-year and the proportion of AI performed in a single date used as threshold for classifying AI as timed AI.
Furthermore, we also tested a method developed by Batista et al. (2016) that uses the discrepancy from an equal number of breedings on each day of the week to identify TAI. In this method, the standard deviation (SD) of the weekly AI for each herd-year is first calculated. Then, if the number of breedings in the day of the week is more than 2 SD plus the mean number of breedings (i.e., 14.3%), breedings that took place on that day are assumed to be synchronized (TAI).

**Data Edit and Definition of Fertility Traits**

To derive fertility traits for this study, we selected data of first-parity cows that calved between 18 and 38 mo of age and data of second-parity cows that calved between 30 and 48 mo of age and had at least one mating recorded following calving. The cows that were selected for this study were pure Holstein and Jersey and their various crosses. All the cows were progeny of Holstein or Jersey bulls.

Mating and calving data were used to define the following fertility traits: CFS, calving interval (CIN), first service nonreturn rate (FNRR), and successful calving rate to first service (SCRFS). The first 3 traits are part of the current Australian multitrait genetic evaluation model, and the fourth trait was included in the study because it is less affected by a possible difference in culling rates between cows in the 2 AI groups. The traits were defined for both TAI and EAI. All cows in this study were included if they had a CFS that ranged from 21 to 321 d. For cows with valid CFS data, the CIN had to range between 290 and 733 d to be valid. Calving intervals of cows below 303 d were set to 303 d, and those longer than 603 d were set to 603 d. Calving intervals below 290 d and above 733 d were set to missing. Cows without a subsequent calving date were assigned a CIN equivalent to their lactation lengths plus 50 d, if they were lactating for at least 280 d. Cows with lactation lengths below 280 d and longer than 700 d were set to missing, and those longer than 553 d up to 700 d were set to 553, which means their CIN was set to 603 d. First service nonreturn rate was coded as 0 if a subsequent breeding was recorded, otherwise 1 (if no service occurred), and was valid for cows that first mated before 2021. For cows that had their FS recently (i.e., 2021) and had not yet been mated again, FNRR was set to missing. Successful calving rate to FS was coded as 1 if the cow calved between 264 and 294 d after the FS. Calving before 264 d from the FS was set to missing unless abortion was recorded as the termination code. For cows that aborted, SCRFS was coded as 1 if the interval from FS to abortion date varied from 100 to 272 d. Cows that calved after 294 d from the FS and those with no subsequent calving when they were present in the herd (i.e., determined by lactation length or termination date) were assumed not to have calved to the service and were coded as 0.

**Data Analyses**

All data analyses were carried out using ASReml (Gilmour et al., 2015). First- and second-parity fertility data of cows were analyzed separately. The model used included the following fixed effects: age at calving (linear and quadratic regression), month of calving, herd-year-season of calving, and the breed of the cow and the bull. The data analyses in this study were performed using sire models to estimate genetic parameters, which included 4 traits to minimize the requirement for huge computational resources and animal models to predict EBV for all animals.

**Estimation of Genetic Parameters.** To meet the objective of the study, genetic parameters for CFS, CIN, FNRR, and SCRFS for TAI and EAI were estimated. For genetic parameter estimation in addition to the fixed effects, the random effect of the sires of the cows with a numerator relationship matrix of their ancestors was also fitted. First, genetic parameters of the 4 fertility traits (i.e., a 4-trait model) based on the data of cows that were categorized as TAI and EAI were estimated separately.

The statistical model in matrix notation can be written as

\[ y_t = X_t b_t + Z_t s_t + e_t, \]  

where \( y_t \) is the observation on all 4 traits, \( X_t \) is the incidence matrix linking the observations to the fixed effects (herd-year-season of calving, age, breed of cow and bull, and month of calving) for trait \( t \), \( b_t \) is the vector of all fixed effects for trait \( t \), \( Z_t \) is incidence matrix linking the observations to the random effects for trait \( t \), \( s_t \) is vector of random sire effects for trait \( t \), and \( e_t \) is the vector of random residual effects for trait \( t \). The expectations (E) are \( E(y_t) = X_t b_t \), \( E(s_t) = 0 \), and \( E(e_t) = 0 \), with the following variance-covariance matrix: \( \text{Var}(s) = G_t \otimes A_s \) for the sire model and \( \text{Var}(e) = R_t \otimes I_e \), where \( G_t \) and \( R_t \) are variance-covariance matrices of size 4. \( G \) is genetic variance-covariance for fertility traits, \( R \) is residual variance-covariance for fertility traits, \( A \) is numerator relationships, and \( I \) is identity matrices.

Second, genetic correlations between CFS, FNRR, SCRFS, and CIN based on TAI and EAI were estimated by assuming traits in each AI group to be different traits. In these models, the residual correlations across
AI groups were assumed to be 0, because cows with fertility data in the TAI group did not have data in the EAI group. These analyses were accomplished using several 4-trait models, by including 2 traits from TAI and 2 traits from EAI at a time because traits recorded in each AI group were considered as different traits. For these analyses, the following genetic and residual variance-covariance matrices were assumed:

\[
G_t = \begin{bmatrix}
\sigma^2_{T_{11}} & \sigma_{T_{12}} & \sigma^2_{T_{12}} & \sigma^2_{T_{22}} \\
\sigma_{T_{12}} & \sigma^2_{T_{12}} & \sigma^2_{T_{12}} & \sigma^2_{T_{22}} \\
\sigma^2_{T_{12}} & \sigma^2_{T_{12}} & \sigma^2_{E_{11}} & \sigma^2_{E_{12}} \\
\sigma^2_{T_{12}} & \sigma^2_{T_{12}} & \sigma^2_{E_{12}} & \sigma^2_{E_{22}} \\
\end{bmatrix},
\]

\[
R_t = \begin{bmatrix}
\sigma^2_{T_{11}} & 0 & 0 & 0 \\
0 & \sigma^2_{T_{22}} & 0 & 0 \\
0 & 0 & \sigma^2_{E_{11}} & 0 \\
0 & 0 & 0 & \sigma^2_{E_{22}} \\
\end{bmatrix},
\]

where \(G_t\) and \(R_t\) are symmetric matrices of the genetic and residual variance-covariance, respectively, and \(T\) and \(E\) are traits 1 and 2 observed in TAI (\(T\)) and in EAI (\(E\)).

**Prediction of Breeding Values.** From the first-parity data, EBV for bulls and cows based on TAI and EAI were calculated to assess mean, SD, and reliability of EBV (calculated from prediction error and genetic variance; e.g., Lynch et al., 2021) and the correlations between EBV. All EBV were calculated using animal models, with the parameters estimated above, for TAI and EAI separately. For the estimation of EBV, animal models with the pedigree of all cows (i.e., cows with TAI and EAI) were used. In this case, the numerator relationship matrix included all cows and their ancestors. All the sires and 95% of the dams of the cows with fertility data in this study were known. The pedigrees used in both cases (sire and animal models) were extracted from the pedigree data that were used for genetic evaluations of Australian dairy cattle by DataGene.

As the reliability and spread of EBV (i.e., SD) are influenced by the quantity and quality of data, EBV were predicted using (1) the data of cows with TAI (Table 1), (2) a randomly selected subgroup of 128,981 cows (equal size as the data of TAI) from the data of cows classified as EAI, and (3) the rest of the data of cows that were classified as EAI (Table 1). Estimated breeding values were calculated using a bivariate model by analyzing CFS with FNRR or CIN or SCRFS.

After EBV were calculated for all traits, the mean, SD, and reliability of EBV from TAI and EAI for bulls and cows were compared. First, for all traits, EBV of bulls with daughters in TAI and randomly selected EAI were compared with EBV from the rest of EAI, assuming that EBV from the larger data set were closer to the true value. For comparing bulls, EBV that were calculated based on randomly selected cows from EAI were used as a benchmark. Second, to assess the extent of bias and usefulness of cow EBV from TAI data, we compared the EBV for these cows to the EBV that were calculated based on data of cows in the rest of EAI. This was done by including the cows classified as TAI in the pedigree without their own data, which was effectively using parent average.

**Results**

**Identification of TAI**

Of the total number of breedings, 0.114 were classified as possibly performed following TAI, based on the proportion of AI that were recorded on a single

<table>
<thead>
<tr>
<th>Trait</th>
<th>TAI</th>
<th>EAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>128,997</td>
<td>750,007</td>
</tr>
<tr>
<td>CIN</td>
<td>110,904</td>
<td>665,377</td>
</tr>
<tr>
<td>FNRR</td>
<td>128,525</td>
<td>749,157</td>
</tr>
<tr>
<td>SCRFS</td>
<td>105,864</td>
<td>636,785</td>
</tr>
<tr>
<td>CFS</td>
<td>97,913</td>
<td>619,409</td>
</tr>
<tr>
<td>CIN</td>
<td>85,027</td>
<td>549,734</td>
</tr>
<tr>
<td>FNRR</td>
<td>97,451</td>
<td>618,531</td>
</tr>
<tr>
<td>SCRFS</td>
<td>80,118</td>
<td>519,825</td>
</tr>
</tbody>
</table>

Table 1. Number of cows with valid records, mean, SD, and median for calving to first service (CFS), calving interval (CIN), first service nonreturn rate (FNRR), and successful calving rate to first service (SCRFS) for AI classified as timed AI (TAI) and estrus-detected AI (EAI) in first and second parities.
Figure 2. The total number of AI by proportion of AI performed on a single date of the year for AI classified as timed AI (TAI) and estrus-detected AI (EAI).
400 matings per year was small, particularly after 2007. Figure 5 shows the proportion of herds that were classified as having TAI relative to the total number of herds in each year from 1995 to 2020. The increase in the number herd-years that possibly used TAI was from about 0.21 (1995) to 0.37 (2020).

When the discrepancy from equal numbers of breedings on each day of the week (Miller et al., 2007) was used to classify AI as following synchronization protocols, the proportion was estimated to be 0.040 and the number of herd-years was 1,237, which represented 0.039 of the herd-years (herd size adjusted χ² of 84 or greater) with 0.533 of AI performed on Thursday and Friday. When a relaxed criterion of χ² (i.e., 45) was used, the total number of AI classified as TAI increased to about 0.9 million (~0.074 of all AI) and the number of herd-years increased to 2,418 (0.076 of all the herd-years). In this case, 0.462 of the AI were performed on Thursday and Friday.

When the approach of Batista et al. (2016) was used to identity AI as TAI, the proportion of AI classified as possibly TAI was 0.045. The total number of herd-years classified as TAI was 3,175, which represents about 0.101 of the herd-years (i.e., 1.3 million AI). In this approach, only the proportion of AI that were carried out on a single day (i.e., higher than a set threshold for the herd-year) were classified as TAI and about 0.257 and 0.185 of the AI were performed on Thursday and Friday, respectively. The total proportion of AI carried out on both days was 0.442, which was the same result obtained when the method proposed in the current study was used to classify AI.

In our study, the proportion of AI on the same date of the year was used to classify AI. The approach appears to be comparable to the other methods that used a deviation from an equal number of breedings on 7 d of the week as the criterion (Miller et al., 2007; Batista et al., 2016) for identification of TAI. The current approach classified 0.612 and 0.656 more AI as being TAI compared with the methods of Batista et al. (2016) and Miller et al. (2007), respectively. This finding indicates a substantial discrepancy between the methods. When the relaxed criterion of adjusted χ² of 45% was assumed, the current approach classified 0.338 more than the method of Miller et al. (2007). Of the total AI classified as TAI using the Batista et al. (2016) method, 0.671 overlapped with ones classified by the approach proposed in the current study. Of the total number of AI classified as TAI using the method of Miller et al. (2007), 0.631 (χ² 84%) and 0.527 (χ² 45%) overlapped
with the ones classified by the approach proposed in this study.  

**Descriptive Statistics**

Table 1 shows the mean, SD, and median for CFS, CIN, FNRR, and SCRFS for first- and second-parity cows with TAI and EAI. Differences between TAI and EAI were small for all traits, with the mean and median for CFS and CIN showing slightly shorter intervals for AI classified as TAI compared with EAI. The difference between TAI and EAI was highly statistically significant ($P < 0.0001$) in both parities for CFS but not for CIN. However, these results mask the slightly lower fertility of AI classified as TAI because 14% of them, compared with 11% classified as EAI, did not recalve, or their approximate CIN from lactation lengths could not be determined. The lower fertility of cows that underwent synchronization was captured when fertility was expressed as SCRFS and FNRR (Table 1) in both parities. Cows with EAI showed 3 and 1% better fertility outcomes than those with TAI when fertility was expressed as SCRFS and FNRR, respectively. The differences in both parities were highly statistically significant ($P < 0.0001$) for both traits.

Figure 6 shows that the distribution of CFS in TAI was narrower than in EAI. The proportion of CFS between 83 and 103 d (i.e., close to the mean or median) was 35% for TAI and 31% for EAI. In the case of CIN, the distribution in TAI cows was also narrower than in EAI cows (data not shown). However, the difference between TAI and EAI was less marked for CIN than for CFS with 27% of the CIN occurring between 355 and 375 d in TAI and 25% in EAI.

**Genetic Parameters**

Table 2 shows phenotypic and genetic variance for each trait in parities 1 and 2 for TAI and EAI. For all traits, the phenotypic and genetic variance were lowest for TAI. The biggest difference in genetic variance was for CFS followed by FNRR and CIN. The difference in phenotypic variance was small, except for CFS for which the phenotypic variance for TAI was about a third of that for EAI. The difference in both genetic and phenotypic variances was smallest for SCRFS.

Table 3 shows heritability, genetic, and residual correlations for CFS, CIN, FNRR, and SCRFS for TAI and EAI in parities 1 and 2. Overall, heritability estimates were lower for CFS, FNRR, and CIN when they
were estimated using TAI in the first parity. In the second parity, the lowest heritability was estimated for CFS. The genetic correlation between CFS and CIN was lower in TAI than in EAI in the first parity (Table 3). In the second parity, the genetic correlation between CFS and CIN was higher in TAI than in EAI (Table 3), simply because the genetic variance for CFS was effectively zero (Table 2). The residual correlations of CFS with CIN and FNRR, which were estimated with higher accuracy (i.e., smaller standard error) than the genetic correlations, show that they were lower in TAI than in EAI (Table 3) in both first and second parities. It is also worth noting that the correlation of CFS with SCRFS had a positive residual and negative genetic correlation in both parities in TAI and EAI. The genetic correlation of CIN with SCRFS was slightly stronger in TAI than in EAI in both first and second parities. All correlations between traits were associated with a larger sampling error in the second parity than in the first parity (Table 3) mainly due to the differences in amount of data (Table 1).

Table 4 shows genetic correlations between a trait in TAI (e.g., CFS) and all traits including CFS in EAI. The values on the diagonals (i.e., genetic correlations of the same trait measured in TAI and EAI) were close to 1 (0.84–0.98) except for CFS, particularly in the first parity (0.55). As expected, the genetic correlations between a trait measured in TAI and another trait measured in EAI were mostly lower than those estimated within the same AI group shown in Table 3. However, the 2 estimates were close to each other except for the genetic correlations of CFS with other traits. It is also worth noting that these correlations were associated with large standard errors and are in Table 4 for completeness.

**Estimated Breeding Values**

Table 5 shows the mean EBV, SD, and reliabilities and correlations between EBV based on TAI and EAI for bulls with at least 50 daughters in the whole data (2,193 bulls), and 150 daughters in the whole data (348 bulls) and 50 daughters in the TAI data. The EBV are for CFS, CIN, FNRR, and SCRFS for bulls based on TAI, randomly selected EAI cows (the same size as TAI), and EAI of first-parity cows (excluding the data of the randomly selected cows). Estimated breeding values for CFS based on TAI showed the lowest
variation and were estimated with the lowest reliability; they also had the lowest correlation with EBV that were calculated based on the rest of EAI (Table 5). Similar trends were also observed when EBV for FNRR were calculated using TAI. The increase in EBV variability and reliability observed in the rest of the EAI compared with the randomly selected EAI data reflects the difference in the size of the data (4.8 times more cows with data). However, the differences between TAI and randomly selected EAI are likely due to the differences in data quality because the data size was the same. The correlations between EBV of the same trait when calculated using data classified as TAI and EAI (the rest of the first-parity data) for bulls with at least 50 daughters in the whole data were lower than those based on bulls with at least 50 daughters in the TAI and 150 daughters in the whole data. As expected, Table 5 shows that the reliability and variability of EBV were higher when bulls with at least 50 daughters in the EAI and at least 150 daughters in the whole data were considered, compared with bulls with at least 50 daughters in the whole data.

Table 6 shows mean EBV, SD, and reliability of cows with TAI calculated using their own data and those based on their relationship to animals with EAI data (i.e., excluding their own data). Estimated breeding values derived from their relationship with the data of the rest of the cows with EAI showed more reliability and variability. The differences were especially in favor of using the bigger data of EAI instead of the data of TAI for CFS EBV. The correlations between EBV using the cows’ own data and the EBV obtained from the use EAI data were lowest for CFS (Table 6) followed by FNRR. Correlations between the EBV obtained from the cows’ own data and that derived through relationships with EAI data were above 0.59 (Table 6) for all traits except CFS.

**DISCUSSION**

One of the objectives of this study was to quantify the extent of synchronization and TAI practices in Australian dairy herds that submitted mating data for routine genetic evaluations of female fertility. Having a large proportion of AI recorded on a single date in the herd-year was assumed to be an indicator of TAI. Accordingly, we estimated that approximately 11% of AI were performed following synchronization. The
### Table 2. Phenotypic and additive genetic variances, with SE, for fertility traits classified as timed AI (TAI) and estrus-detected AI (EAI) in first and second parities

<table>
<thead>
<tr>
<th>Trait</th>
<th>Phenotypic</th>
<th>Genetic</th>
<th>Phenotypic</th>
<th>Genetic</th>
<th>Phenotypic</th>
<th>Genetic</th>
<th>Phenotypic</th>
<th>Genetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>442.2 ± 1.8</td>
<td>3.6 ± 1.1</td>
<td>1,327.0 ± 2.3</td>
<td>30.1 ± 2.2</td>
<td>454.3 ± 2.2</td>
<td>1.1 ± 0.9</td>
<td>1,345.7 ± 2.5</td>
<td>27.6 ± 2.3</td>
</tr>
<tr>
<td>CIN</td>
<td>4,339.4 ± 19.4</td>
<td>81.6 ± 15.0</td>
<td>4,778.4 ± 8.7</td>
<td>166.8 ± 10.4</td>
<td>4,459.8 ± 24.0</td>
<td>121.4 ± 22.3</td>
<td>4,926.6 ± 10.2</td>
<td>151.4 ± 11.2</td>
</tr>
<tr>
<td>FNRR</td>
<td>2,065.0 ± 8.5</td>
<td>14.7 ± 4.2</td>
<td>2,129.8 ± 3.6</td>
<td>24.6 ± 2.3</td>
<td>2,064.2 ± 9.9</td>
<td>20.6 ± 6.2</td>
<td>2,129.9 ± 4.0</td>
<td>23.1 ± 2.4</td>
</tr>
<tr>
<td>SCRFS</td>
<td>2,238.8 ± 10.0</td>
<td>26.2 ± 5.7</td>
<td>2,262.3 ± 4.1</td>
<td>34.5 ± 2.8</td>
<td>2,261.3 ± 12.0</td>
<td>27.3 ± 7.6</td>
<td>2,290.4 ± 4.5</td>
<td>29.2 ± 2.8</td>
</tr>
</tbody>
</table>

1CFS = calving to first service; CIN = calving interval; FNRR = first service nonreturn rate; SCRFS = successful calving rate to first service.

### Table 3. Genetic parameters (heritability on the diagonal, genetic correlation above, residual correlation below diagonal; ±SE) for fertility traits classified as timed AI (TAI) and estrus-detected AI (EAI) in first and second parities

<table>
<thead>
<tr>
<th>Trait</th>
<th>CFS</th>
<th>CIN</th>
<th>FNRR</th>
<th>SCRFS</th>
<th>CFS</th>
<th>CIN</th>
<th>FNRR</th>
<th>SCRFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>0.01 ± 0.00</td>
<td>0.40 ± 0.15</td>
<td>−0.02 ± 0.22</td>
<td>−0.16 ± 0.19</td>
<td>0.02 ± 0.00</td>
<td>0.72 ± 0.03</td>
<td>−0.02 ± 0.06</td>
<td>−0.32 ± 0.05</td>
</tr>
<tr>
<td>CIN</td>
<td>0.22 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>−0.82 ± 0.11</td>
<td>−0.99 ± 0.04</td>
<td>0.38 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>−0.63 ± 0.00</td>
<td>−0.87 ± 0.02</td>
</tr>
<tr>
<td>FNRR</td>
<td>0.07 ± 0.00</td>
<td>−0.34 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.88 ± 0.07</td>
<td>0.12 ± 0.00</td>
<td>−0.31 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.90 ± 0.02</td>
</tr>
<tr>
<td>SCRFS</td>
<td>0.04 ± 0.00</td>
<td>−0.55 ± 0.00</td>
<td>0.67 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>−0.51 ± 0.00</td>
<td>0.68 ± 0.00</td>
<td>0.02 ± 0.00</td>
</tr>
</tbody>
</table>

1CFS = calving to first service; CIN = calving interval; FNRR = first service nonreturn rate; SCRFS = successful calving rate to first service.

Estimates or their standard errors are rounded to zero.
proportion of AI classified as TAI increased over time and as the number of AI per herd increased (which was assumed to be a measure of herd size). These results generally agree with the result of a survey recently carried out by Dairy Australia (S. Bullen, Dairy Australia, Melbourne, Australia, personal communication) that synchronization has increased over time and is more common in larger herds. The fact that 0.44 of the AI categorized as TAI were performed on 2 d of the week (Figure 3) also agrees with Miller et al. (2007) and Batista et al. (2016), who used the deviation from equal number of breedings on each day of the week as an indicator of synchronization practices.

When the data of AI classified as TAI were used for estimating genetic parameters and genetic evaluations, CFS was the trait most affected. The low phenotypic and additive genetic variance (Table 2), heritability estimates (Table 3), and variability and reliability of EBV for bulls (Table 5) and cows (Table 6) for CFS based on TAI demonstrated that reproductive intervention reduces the usefulness of this trait for genetic evaluation. This outcome generally agrees with that of Lynch et al. (2021), who worked on fertility data from Canadian dairy herds. For the other traits, genetic correlations between a trait measured in TAI and EAI were close to 1 (Table 4). The smaller genetic variance and heritability for FNRR and CIN in TAI compared with EAI suggest that synchronization reduces variation in these traits also.

In this study, we used 3 different methods for classifying AI, but objectively comparing the methods was beyond the scope of the current study owing to the lack of validation data. However, it is important to mention the main differences between the approaches. The

<table>
<thead>
<tr>
<th>TAI trait</th>
<th>EAI trait</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>CIN</td>
</tr>
<tr>
<td><strong>First parity</strong></td>
<td><strong>CFS&lt;sub&gt;TAI&lt;/sub&gt;</strong></td>
</tr>
<tr>
<td>0.55 ± 0.12</td>
<td>0.29 ± 0.12</td>
</tr>
<tr>
<td><strong>Second parity</strong></td>
<td><strong>CFS&lt;sub&gt;TAI&lt;/sub&gt;</strong></td>
</tr>
<tr>
<td>0.88 ± 0.24</td>
<td>0.65 ± 0.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Trait</th>
<th>Data of synchronized cows</th>
<th>Randomly selected with equal size to synch</th>
<th>Data of rest of first-parity cows&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SD</td>
<td>Rel</td>
<td>Correlation&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Bulls with progeny ≥50 in the whole data (n = 2,193 bulls)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFS</td>
<td>0.14</td>
<td>0.02</td>
<td>0.17</td>
</tr>
<tr>
<td>CIN</td>
<td>4.64</td>
<td>5.52</td>
<td>0.25</td>
</tr>
<tr>
<td>FNRR</td>
<td>−0.97</td>
<td>1.65</td>
<td>0.16</td>
</tr>
<tr>
<td>SCRFS</td>
<td>−2.10</td>
<td>2.66</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Bulls with progeny ≥150 in the whole data and at least 50 progeny in synchronized cows (n = 348 bulls)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFS</td>
<td>0.17</td>
<td>0.86</td>
<td>0.33</td>
</tr>
<tr>
<td>CIN</td>
<td>5.89</td>
<td>7.01</td>
<td>0.48</td>
</tr>
<tr>
<td>FNRR</td>
<td>−1.33</td>
<td>2.24</td>
<td>0.30</td>
</tr>
<tr>
<td>SCRFS</td>
<td>−2.71</td>
<td>3.42</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<sup>1</sup>CFS = calving to first service; CIN = calving interval; FNRR = first service nonreturn rate; SCRFS = successful calving rate to first service.

<sup>2</sup>Correlation of EBV with corresponding EBV predicted based on data from the rest of first-parity estrus-detected cows.

<sup>3</sup>Data of EAI excluding the randomly selected cows.
method of Miller et al. (2007) classifies all herd-years into either TAI or EAI, while our approach classifies AI performed on a single day of the year into TAI, which is suitable for herd-years in which both TAI and EAI are practiced. However, if the objective is to identify herd-years that performed any synchronization, then the approach of Miller et al. (2007) may be more appropriate. The other difference is that the current approach used breeding data of each day of the year for classification of AI, while Miller et al. (2007) and Batista et al. (2016) used data summarized into 7 d of the week (i.e., a summary of 52 d over a year).

Overall, the 3 approaches are likely to suffer from both specificity and sensitivity problems and are only indirect indicators of the use of estrus synchronization protocols. In addition, all approaches could only identify the most obvious anomalies in recording breeding dates (e.g., using generic or default date), which may not have been related to synchronization. The data used for this study were recorded over a period of 25 years, which means the effectiveness of the classification may have been influenced by the level and effectiveness of the synchronizations practiced (e.g., Lucy, 2019), as well as anomalies (pattern) in recording of breeding dates, which may have changed within the same herd and across different herds over the years. For example, in the earlier years (i.e., 1995–2000), most of the AI classified as TAI could likely be associated with a pattern of recording of breeding date (e.g., entering of matings that took place on several dates as if they were performed on the same date) rather than TAI. However, both have the same effect and make CFS an ineffective measure of fertility.

The fact that the proportion of cows classified as TAI (Figure 4) was smaller than the proportion of herds that used TAI (Figure 5) indicates that TAI was used on a subset of possible problem cows within the herd, rather than on all cows in the herd. This practice is in general agreement with the recommendations in Australia where selective synchronization of cows would lead to better conception outcomes (Shephard et al., 2014). The trends are similar to those reported for Canadian dairy herds in which breeding method was recorded (Lynch et al., 2021). In Canadian dairy herds, the percentage of herds that used TAI has increased from 30% to 59%, whereas the percentage of cows that were synchronized increased from 12% to almost 18% over a decade.

Efficiency in reproductive performance measured as CFS appeared to be better in TAI than in EAI (Table 1). However, when reproductive performance was measured as FNRR and SCRFS, cows inseminated following synchronization appeared to have a lower fertility outcome. This result conflicts with a report by Ungerfeld et al. (2021), who observed slighter greater pregnancy rates than for insemination following detected estrus. The result of this study regarding fertility outcomes such as FNRR, SCRFS, and CIN should be interpreted with caution and could be affected by possible misclassification of AI due to recording anomalies more than CFS. Some evidence suggests that cows subjected to reproductive intervention are those with reduced fertility (e.g., Lynch et al., 2021). In Australia, as was also observed in Canada (Lynch et al., 2021), synchronization may be practiced as a last resort to increase the probability of pregnancy with the aim of culling cows that do not get pregnant following synchronization or mating them to natural service bulls (A. Daniel, Bull Nu-Genes, Terang, Victoria, Australia, personal communication). The lower number of cows that recalved and the lower FNRR and SCRFS in TAI than in EAI (Table 1) support this observation. Second, as we used the proportion of breeding on each day of the year as an indicator of TAI, a misclassification of AI may affect traits associated with fertility outcomes without affecting their CFS. The efficiency of reproductive interventions with regard to fertility outcomes varies (Lucy, 2019) owing to the difference in the protocols and their

<table>
<thead>
<tr>
<th>Trait</th>
<th>EBV based on TAI (i.e., own data)</th>
<th>EBV based on relationship to EAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>CFS</td>
<td>0.14</td>
<td>0.48</td>
</tr>
<tr>
<td>CIN</td>
<td>4.44</td>
<td>1.26</td>
</tr>
<tr>
<td>FNRR</td>
<td>−0.98</td>
<td>1.98</td>
</tr>
<tr>
<td>SCRFS</td>
<td>−2.00</td>
<td>1.98</td>
</tr>
</tbody>
</table>

1CFS = calving to first service; CIN = calving interval; FNRR = first service nonreturn rate; SCRFS = successful calving rate to first service.
2Correlation of EBV obtained from the data with corresponding breeding values predicted based on the relationship for cows with data.
effectiveness, as well as the inherent fertility of the cows (e.g., age, milk production, BCS) that undergo synchronization.

Genetic parameters, including heritabilities estimated using data currently available, are similar to the previous results estimated based on Australian data (e.g., Haile-Mariam et al., 2004; Morton et al., 2018) and are also within the range of estimates in several other countries (e.g., Fleming et al., 2019). However, our estimates are lower than those estimated by Lynch et al. (2021) for both TAI and EAI using Canadian data. The heritabilities of days open reported by Lynch et al. (2021), which varied between 0.025 and 0.037, were similar to our estimate for CIN (Table 3). In general, the observation that heritabilities for different traits were lower when estimated using TAI data than EAI data agrees with that of Lynch et al. (2021). The residual variance for CFS in TAI being around a third of that for EAI agrees with the observations of Miller et al. (2007), who reported that CFS of confirmed TAI was 38% of that in EAI data. Goodling et al. (2005) reported that residual variance for CFS was more affected by breeding method than sire variance, which means few differences in heritability existed between breeding methods.

The difference in phenotypic and genetic variance (Table 2), heritability (Table 3), and SD and reliability of EBV of CFS between TAI and EAI (Tables 5 and 6) observed in the current study is likely to be higher than that calculated in the current routine genetic evaluation of fertility. The reason for this possibility is that the data analyses were performed separately for the 2 AI groups. In the current routine genetic evaluation in Australia, where data of both AI groups are analyzed together, any differences in EBV of bulls with daughters in both AI groups is small because most of the bulls will have most of their daughters in the EAI group. For cows with TAI data, their EBV may be regressed more toward the mean (i.e., zero) compared with EBV of cows with EAI (Table 6). Table 6 also shows that for cows with TAI data, using pedigree information provides a more realistic EBV than using the cows’ own data because the heritability of CFS of TAI is effectively zero. The very low heritability of CFS from TAI data and the overall low heritability of all fertility traits means that quantifying bias on EBV of even cows with their own TAI data is difficult because the use of the cow’s own record may add more noise rather than introduce bias that can be quantified (Oliveira Junior et al., 2021). Furthermore, EBV of cows are more likely to be affected by TAI because their own affected data are used, compared with bulls for which data of their TAI and EAI progeny are possibly used.

The results of this study agree with those of Lucy (2019) and Lynch et al. (2021) in that CFS is not effective for measuring variation in fertility of dairy cows that undergo synchronization. In countries such as Australia where both TAI and EAI are practiced simultaneously, identifying treated cows is necessary if CFS is going to continue to be part of the multitrait genetic evaluation model. In this case, the CFS of cows with TAI can be set to missing in the multitrait model and the data of unaffected cows can continue to be used for genetic evaluations. Similarly, CFS data of herd-years with synchronization can be set to missing. However, both require an identification of AI administered following reproductive intervention because breeding methods are not recorded. Alternatively, CFS, which is the trait that is most affected by synchronization, can be excluded from the multitrait model, and EBV for fertility traits can be calculated based on measures such as calving interval, FNRR, lactation length, and pregnancy rate (e.g., Haile-Mariam and Pryce, 2012). The benefit of this is that classifying AI is unnecessary. In Australia, although CFS (i.e., mating data) is available on about a third of the cows used for genetic evaluation of fertility, it is also a useful early indicator of fertility, particularly in seasonal calving systems (Grosshans et al., 1997; Haile-Mariam et al., 2004). The inclusion of CFS as one of the fertility traits in the multitrait genetic evaluation is also useful because on average about 11.7% the cows with CFS do not have actual CIN or predicted CIN possibly because they do not recalve (due to culling). Therefore, the approach that involves the minimum change to the current genetic evaluation could be to set the CFS data of treated cows to missing. This approach can affect up to 17% of the data (Figure 4) as opposed to setting herd-years (Figure 5) with TAI to missing, which may affect up to 37% of the data.

CONCLUSIONS

Using the proportion of breedings performed on a single day of the year in a herd as an indirect indicator of synchronization, we found that 0.11 of breedings possibly involved TAI. The phenotypic and additive genetic variance, heritability, and reliability and variability of EBV for bulls and cows for CFS were lower in TAI than those for EAI. For CIN, FNRR, and SCRFS, the genetic correlation between a trait measured in TAI and EAI was close to 1. The lower genetic variances and heritabilities for FNRR and CIN in TAI than in EAI suggest that synchronization reduces variation between animals. In conclusion, synchronization of estrus makes CFS an ineffective measure of fertility. One approach to minimize this effect on genetic evalu-
ations is to identify TAI and set CFS of these cows to missing when running multitrait genetic evaluations of fertility traits that include CFS. In the long term, the most practical and accurate way to reduce the effect of synchronization on genetic evaluations is to record breeding methods along with mating data.

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REFERENCES


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