



Fertility of dairy cows milked once daily or twice daily in New Zealand

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ABSTRACT

The objective of this study was to evaluate the reproductive performance of New Zealand dairy cows with different milking regimens. A total of 2,562 herds calving in 2017 met the criteria for inclusion in this study. The herds were classified into 5 different milking regimens: 260 herds with cows milked once daily (OAD) during the entire lactation, 1,206 herds with cows milked twice daily (TAD) during the entire lactation, 94 herds that were switched to OAD milking from TAD milking during the mating period (OAD-M), 700 herds that were switched to OAD milking from TAD milking after peak lactation (OAD-P), and 302 herds that switched to OAD milking from TAD milking at end of the lactation (OAD-E). Time from the start of mating to first service (SMFS), start of mating to conception (SMCO) and first service to conception (FSCO) were analyzed using survival analysis. Time from SMFS, SMCO and FSCO was significantly shorter in cows milked OAD compared with cows milked TAD. Also, cows milked OAD had fewer services per conception and higher mean 3-wk submission (SR21), in calf by 3 wk (PR21), in calf by 6 wk (PR42), conception to the first service (PRFS), 3-wk calving (CR21) and 6-wk calving (CR42), and lower not in calf (NIC) than herds with TAD, OAD-M, OAD-P, OAD-E milking cows. Fertility performance differed with parity; first-parity cows had lower SR21, 6-wk submission (SR42), PR21, PR42, PRFS, CR21, and CR42 values, and higher NIC values than second-parity cows. Third parity cows had the highest values for SR42, PR21, PR42, PRFS, CR21, and CR42, and lowest value for NIC compared with cows of other parities. Significant but minor interactions between milking regimen and parity existed for SMFS, SMCO, FSCO, SR21, SR42, PR21, PR42, PRFS, NIC, and CR21.

Key words: fertility, milking frequency, crossbreeding, grazing

INTRODUCTION

The New Zealand dairy system is based on seasonal calving, pasture-based dairy farming, with the majority of herds calving in spring. The goal of the reproductive management program is to achieve the highest pregnancy rate as soon as possible after a planned start of mating (PSM) date to maintain the concentrated calving pattern in the next season (Xu et al., 1997). The percentage of cows conceived during the first 42 d (6 wk in calf; PR42) after PSM is considered as a key reproductive measure in a seasonal pasture-based dairy system (Xu and Burton, 2003). A reliable estimate for PR42 can be obtained from the results of early aged pregnancy testing (Hemming et al., 2018). Xu and Burton (2003) reported that of the key fertility traits, PR42 had the greatest variation in herd-level performance in New Zealand. Top quartile herds achieved 78% PR42, the bottom quartile 57%, indicative of an effect of farm management on reproductive outcomes in New Zealand dairy herds.

Poor reproductive performance leads to a delay in the mean calving date, a less compact calving pattern and a reduced lactation length in the next season (Macmillan, 1979). Failure to conceive is a major cause of culling in New Zealand herds: of cows that were culled, 33.4% of them were culled for being not in calf (NIC; Kerslake et al., 2018). The dairy industry in New Zealand has targets for fertility performance (Burke and Fowler, 2007), but for the production year 2017-2018, the national averages of the fertility measures were below the industry targets (Table 1).

Milking frequency is recorded on herd test dates, providing a mechanism to determine the proportion of herds using different milking regimens. In the production year 2015-2016, about 9% of herd-tested herds milked once daily (OAD) for the whole lactation in New Zealand (Edwards, 2018). Farmers cite several benefits from changing to OAD milking. These include ease of attracting labor, reduced labor costs, management of feed shortfalls, herd expansion, allowing time to build capital, utilizing farmland with hilly terrain or long walking distances, herd health, and management

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Table 1. Description of fertility traits and target values of New Zealand dairy industry and national averages reported in production year 2017–2018

Trait	Criteria for trait calculation ¹	Target, ² %	National average, ³ %
SMFS	The interval from the start of mating to the first service (d)		
SMCO	The interval from the start of mating to conception (d)		
FSCO	The interval from the first service to conception (d)		
SC	Number of services for conception (count)		
SR21	Cows with the first mating date in the first 21 d from the start of mating date were represented as 1, otherwise coded as 0 (binary)	90	79.1
SR42	Cows with the first mating date in the first 42 d from the start of mating date were represented as 1, otherwise coded as 0 (binary)		
PR21	Cows conceived in the first 21 d from the start of mating date represented as 1, otherwise coded as 0 (binary)		
PR42	Cows conceived in the first 42 d from the start of mating date represented as 1, otherwise coded as 0 (binary)	78	66.6
PRFS	Cows conceived to the first AB insemination represented as 1, otherwise coded as 0 (binary)	60	54.0
NIC	Cows not in calf at the end of the mating period represented as 1 and in calf cows were coded as 0 (binary)	8	16.8
CR21	Cows calved in the first 21 d from PSC date represented as 1, otherwise coded as 0 (binary)	60	
CR42	Cows calved in the first 42 d from the PSC date represented as 1, otherwise coded as 0 (binary)	87	

¹AB = artificial breeding; PSC = planned start of calving.

²Source: Burke and Fowler (2007).

³Source: DairyNZ (2020).

(Bewsell et al., 2008). Furthermore, OAD milking can reduce the extent of negative energy balance (**NEB**) that occurs after parturition (McNamara et al., 2008; Phyn et al., 2010; Kay et al., 2013).

Research on the effect of strategic OAD milking on cow fertility has been limited. Only 3 studies compared the fertility of cows milked the entire season OAD and TAD in New Zealand (Clark et al., 2006; Edwards, 2018; Hemming et al., 2018). In an experimental study, Clark et al. (2006), reported cows milked OAD conceived earlier with a greater (>7.3%) 3-wk submission (**SR21**) and a greater (>7.8%) 3 wk in calf (**PR21**) than cows milked TAD. Hemming et al. (2018) and Edwards (2018) used data from commercial herds to compare the fertility performance of herds milked OAD or TAD. Percentages of cows that calved in the first 3 wk (**CR21**) and 6 wk (**CR42**) of the subsequent calving season were 5% higher in herds milked OAD compared with herds milked TAD (Edwards, 2018). Hemming et al. (2018) investigated the reproductive performance of OAD (n = 75) and TAD (n = 76) milking herds from season 2014 to 2016 in New Zealand. Herds milked OAD had better fertility performance; a 7.7% higher mean SR21, 7.9% higher mean conception to first service, 10.4% higher mean PR42, 4.8% lower mean NIC, and 4.3% more of the herd calved by 6 wk of calving than TAD milking herds. However, still, there is a paucity of information on interval fertility traits of cows milked under OAD and no information of the effect of herds that milk OAD for some portion of the lactation on fertility performance. Switching to OAD milking after peak lactation or in late lactation

are common practices in New Zealand dairy farming as a preference for farmers' lifestyle and management of available feed during dry summer conditions. However, fertility outcomes associated with these milking practices have not been investigated.

Parity effects on reproductive performance in lactating cattle have been reported in New Zealand. Grosshans et al. (1997) reported that the second-parity cows had better reproductive performance than first-parity cows and had shorter intervals from the start of mating to first service (**SMFS**), the start of mating to conception (**SMCO**) and higher PR21 and PR42. Xu and Burton (2003) reported that age and parity affected reproductive performance. Poorer reproductive performance was identified in 2-yr-old (first parity) and ≥8-yr-old cows (predominantly ≥7 parity) compared with 3- to 6-yr-old cows (predominantly second to fifth parity). Understanding the effect of parity on reproductive performance can help farmers identify cows that need additional management attention. The objective of this study was to evaluate the reproductive performance of New Zealand dairy cows with different milking regimens and parity number.

MATERIALS AND METHODS

Data

Data on herd test milk yields, calving, mating and pregnancy diagnosis dates, and breed information of spring-calved dairy cows in the production year 2017–2018 (June 1, 2017 to May 31, 2018) were extracted

from Livestock Improvement Corporation (**LIC**), New Zealand. Initial data sets contained data from 4,173 herds. Initial screening removed herds that were autumn calving or split calving (calving in both autumn and spring), or that had fewer than 50 cows, leaving 3,823 herds. Herds with fewer than 4 herd tests in the 2017-2018 production year were removed. Herds without artificial breeding (**AB**) records for at least 50% of the cows were also removed, leaving a total of 3,725 herds.

Classification of Herds

Herd test dates and information of daily milk yields were used to determine the milking frequency at each stage of the lactation for each cow. If more than 90% of tested cows at a herd-testing date were milked OAD, then the herd was identified as OAD milking herd on that herd test date. Likewise, if more than 90% of cows at a herd test date were recorded as TAD, then it was identified as a TAD milking herd at that herd test date. Herds that could not be classified as either OAD or TAD at a particular herd test were excluded. If all the herd tests in a season for a herd were classified as OAD, the herd was coded as a full season OAD milking herd, similarly for TAD herds. In addition, herds switching to OAD milking from TAD milking for a specific period of the lactation were identified. These categories were OAD milking during the mating period (**OAD-M**), OAD milking after peak lactation (**OAD-P**), and OAD milking in late lactation (**OAD-E**).

The first herd test usually occurs within the first 60 d after herd's planned start of calving (**PSC**) date, the second usually between 120 d from the first herd test and 180 d from the PSC. The PSC date for each herd in year 2017 was calculated by adding 282 d to herd's mating start date in year 2016. Therefore, either the first or the second herd test can be the closest to the mating period start date, which is around 83 d after the PSC. The average number of herd tests per herd was 4.3. Mating end dates for herds were extracted from the LIC database.

The mating end date of the herd was defined as either the last recorded mating date on or before the 21st wk of the mating period or the last date with 2 conceptions followed by at least 30 d with no conceptions on or before the 21st wk of the mating period or the last date with one conception on or before the 21st wk of the mating period followed by at least 30 d with no conceptions and had at least one conception on each of 2 or more other days in the 6 preceding days. If a herd test was performed before the last mating date for a herd and cows were milked OAD in that herd test, it

was classified as OAD-M. In some cases, 2 herd tests were completed before the mating period finished and cows were milked TAD at the first test date (before the mating period), but OAD at the second herd test during the cows mating period, in such cases those herds were classified as OAD-M. Herds were classified as OAD-P if all cows were milked TAD at herd tests before December 25, 2017, and milked OAD for all herd tests after this date. Herds were classified as OAD-E, if all herd tests were TAD except the last herd test, which was OAD and dated between March and May 2018, and at least one TAD herd test occurred after December 25, 2017. Herds that could not be classified as either OAD, TAD, OAD-M, OAD-P, or OAD-E were excluded from the data set ($n = 907$).

After the OAD and OAD-M herds had been identified, geographic location was used to select TAD, OAD-P, and OAD-E, within 20 km from OAD, OAD-M herds using a GPS visualizer (Schneider, 2012). Some herds classified as OAD, and OAD-M were surrounded by several herds in the TAD, OAD-P, and OAD-E categories. In these cases, all those herds were selected for the study. If OAD or OAD-M milking herd did not have at least one TAD, OAD-P, or OAD-E herd within 20 km, then it was excluded ($n = 2$). Similarly, TAD, OAD-P, and OAD-E herds that were more than 20 km from the nearest OAD or OAD-M herd were excluded ($n = 245$). This left 2,571 herds: OAD (261, 10.2%), TAD (1,214, 47.1%), OAD-M (94, 3.7%), OAD-P (700, 27.2%), OAD-E (302, 11.8%).

Breed Information

A data set with breed composition (expressed in sixteenths) for each cow was used to classify the cows into 3 breed categories; Holstein-Friesian (**F**), Jersey (**J**), and crossbred of $F \times J$. Cows with less than 100% known breed proportions known were removed (101,226). Cows with more than 12.5% of any breed other than F or J were excluded (26,827). Cows (371,517) were classified as F or J if they had breed compositions of $F \geq 14/16$ or $J \geq 14/16$, respectively. Remaining cows were classified as crossbred of $F \times J$. After the breed classification, one OAD and 8 TAD milking herds (1,688 cows) were removed from previously selected herds as they did not have any F, J or $F \times J$ cows.

Proportion of F and J in each cow was calculated with the following equation:

$$\alpha_i^P = \frac{(\alpha_i^S + \alpha_i^D)}{2},$$

where i is breed F or J; and α_i^P is the proportion of genes from breed i in the cow; and α_i^S and α_i^D are the proportions of breed i in the sire and dam, respectively.

Heterosis coefficient for each cow was calculated using the following equation (Dickerson, 1973):

$$h_{F \times J} = \alpha_F^S \alpha_J^D + \alpha_J^S \alpha_F^D,$$

where $h_{F \times J}$ is the coefficient of heterosis between F and J in the progeny; and α_F^S and α_J^S are breed proportions of F and J in the sire; and α_J^D and α_F^D are breed proportions of J and F in the dam, respectively.

Parity

Parity was calculated based on the number of previous calving dates for the cow, with the first 4 parities considered separately and cows in parity 5 and above grouped into one class (parity ≥ 5).

Reproduction Data Set

Cows that did not calve between June 1, 2017, and December 31, 2017, were excluded from the data set, along with cows that had less than 100% recorded breed composition, or greater than 12.5% of breeds other than F or J in their breed composition, leaving 837,212 cows in 2,562 herds. Similarly, cows with no pregnancy diagnosis (PD) record and no herd test records after the end of the mating period were considered to have left the herd before the end of mating and were excluded from the data set ($n = 26,055$), for all traits except SMFS. This resulted in the fertility data set containing 811,157 cows to be considered in this analysis, except for the fertility trait SMFS, which included 837,212 cows.

These cows existed in 2,562 herds: OAD (67,037; 8.3%), TAD (409,474; 50.5%), OAD-M (28,488; 3.5%), OAD-P (201,725; 24.9%), and OAD-E (104,433; 12.9%). The population consisted of 252,589 (31.2%) F, 106,565 (13.1%) J, and 452,003 (55.7%) F \times J cows. The distribution of cows by parity was first (189,274; 23.3%), second (158,493; 19.5%), third (125,856; 15.5%), fourth (105,243; 13.0%), and fifth or greater (232,290; 28.6%).

Fertility Traits

The 12 fertility traits investigated in the present study are defined in Table 1. In the calculation of fertility traits, mating start date was defined as the first of 2 consecutive days with at least one AB insemination or natural service, where at least 3 of the next 6 d also had

mating records. The PSC date for 2018 was calculated for a herd as mating start date in year 2017 plus 282 d. Any mating dates recorded outside the identified mating period for each herd were excluded from the analysis. Pregnancy testing was usually conducted using transrectal ultrasound, but rectal palpation might also have been used. The type of testing is not recorded in the LIC database.

Figure 1 details the calculation of date of conception, 21-d pregnancy outcome (PR21), 42-d pregnancy outcome (PR42) and end-of-mating pregnancy outcome (NIC). Note that induction of parturition is not permitted in New Zealand, so conception dates can be estimated from calving dates. Submission in the first 21 or 42 d of mating (SR21 or SR42) was calculated for all cows in the fertility data set and coded as 0 for cows that did not have an AB record in the first 21 or 42 d of mating, respectively, and 1 for cows that had at least one AB record in the relevant period. Pregnant to first service (PRFS) was only calculated for cows whose first service was to AB and was coded as 1 for cows where date of first service equaled date of conception, and 0 otherwise. Services per conception (SC) was calculated only for cows that conceived to AB ($n = 587,688$) and was the number of AB inseminations recorded for that cow.

Calving by 3 wk and 6 wk in subsequent season were calculated only for cows that had a calving date recorded in 2018 ($n = 493,464$). If the 2018 calving date was less than 21 or 42 d after the PSC in 2018, cow was recorded as calved in 21 (CR21) or 42 d (CR42), respectively. For cows that calved more than 21 or 42 d after the PSC in 2018, CR21 or CR42 was recorded as 0.

Statistical Analysis

All statistical analyses were undertaken using SAS version 9.4 software (SAS Institute Inc.). Binary fertility traits were analyzed using the GLIMMIX procedure with logit link and binomial distribution. The model included the fixed effects of milking regimen, breed, parity, interaction of milking regimen and breed, interaction of milking regimen and parity, linear and quadratic effects of the interval from calving (June 1 to December 31, 2017) to mating start date as covariates, and the random effects of herd and residual. When analyzing NIC, the duration of the mating period length was also fitted as a fixed effect. Least squares means under the logit scale for each level of the fixed effect were obtained and used for multiple mean comparisons using Fisher's least significant difference and then back-transformed into the nominal (binary) scale for

presentation and interpretation. Significant differences between means were declared at $P < 0.05$. Number of SC was analyzed using GLIMMIX procedure with the same model described above, except with log link and Poisson distribution.

Cox proportional hazard models (Proc PHREG) with right-censored intervals were used to analyze

time to event data. Three interval traits were right-censored: SMFS, SMCO and interval from first service to conception (**FSCO**) for cows that did not experience the events. Cows with no recorded AB inseminations (72,059), were right-censored on the date of the end of AB period in each herd. The end of the AB period was defined for each herd as the date of the last

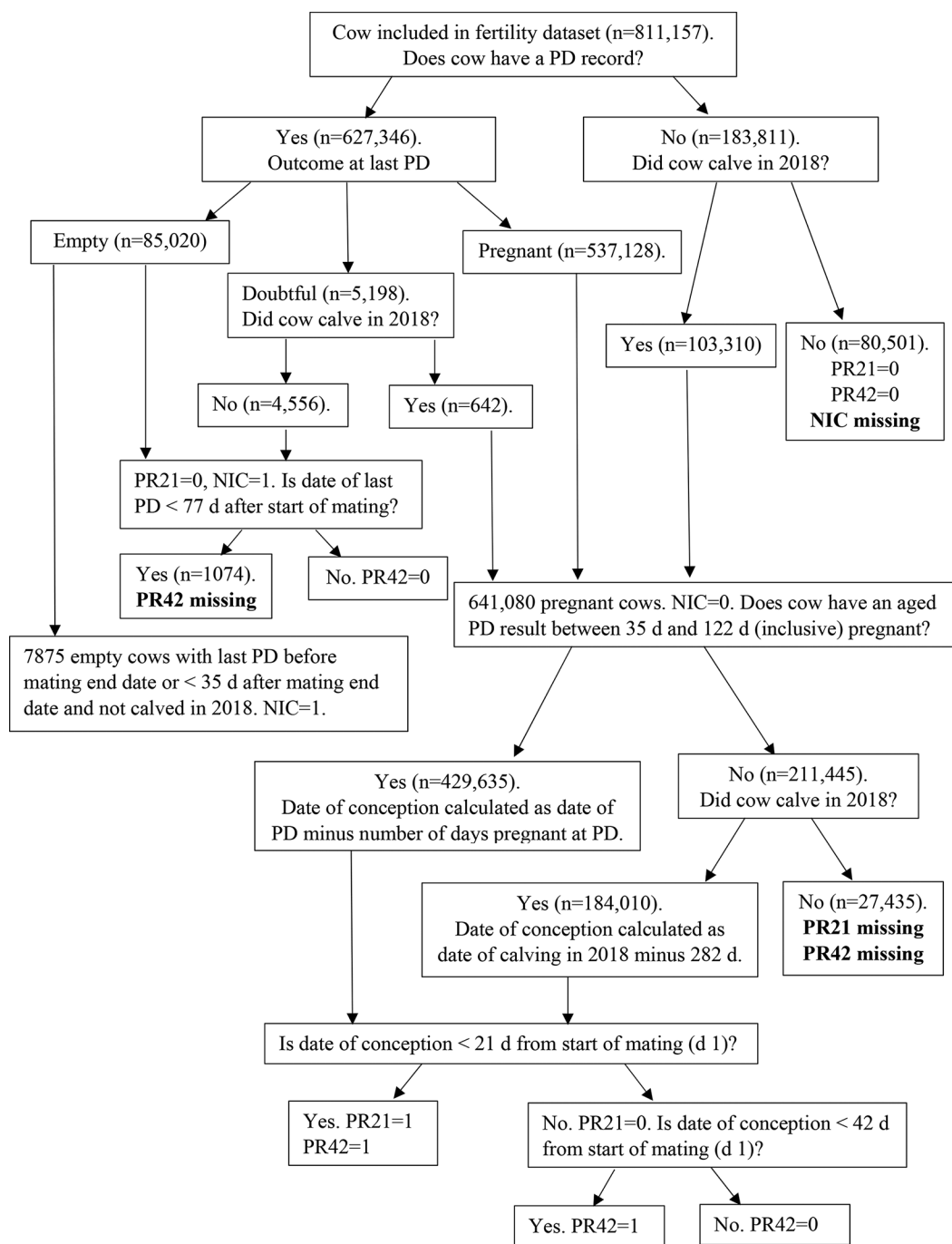


Figure 1. Flowchart detailing determination of date of conception, 21-d pregnancy outcome (PR21), 42-d pregnancy outcome (PR42), and end-of-mating pregnancy outcome. NIC = not in calf; PD = pregnancy diagnosis.

Table 2. Herd characteristic means (SD) according to milking regimen

Characteristic	Milking regimen ¹				
	OAD	TAD	OAD-M	OAD-P	OAD-E
Herd level					
Herd size (cows/herd)	268 (191)	349 (217)	315 (183)	299 (177)	356 (265)
Mating length (d)	69.7 (9.0)	76.2 (10.0)	75.6 (8.8)	73.9 (8.6)	75.0 (10.4)
Start of calving to start of mating date (d) in 2017	83.6 (2.7)	83.1 (2.9)	84.0 (3.1)	83.3 (3.1)	83.1 (2.9)
Production					
Milk yield (kg)	3,201 (860)	4,860 (1,368)	3,957 (1,273)	4,110 (1,071)	4,669 (1,230)
Fat yield (kg)	169 (45)	231 (61)	199 (62)	206 (51)	226 (57)
Protein yield (kg)	131 (34)	186 (50)	156 (48)	161 (40)	183 (47)
Lactose yield (kg)	158 (43)	243 (69)	197 (64)	205 (53)	234 (62)
Breed proportion²					
F proportion	0.46 (0.33)	0.66 (0.31)	0.59 (0.32)	0.56 (0.32)	0.61 (0.31)
J proportion	0.53 (0.33)	0.33 (0.31)	0.40 (0.32)	0.43 (0.32)	0.38 (0.31)

¹OAD = full season once daily milking; TAD = full season twice daily milking; OAD-M = once daily milking in cow's mating period; OAD-P = once daily milking after peak lactation; OAD-E = once daily milking at end of the lactation.

²F = Holstein-Friesian; J = Jersey.

AB insemination that was not followed by another AB inseminations within 7 d. For the analysis of SMFS, cows with no PD and no herd tests records after end of the mating period were right-censored on the last AB date if they had AB records or were right-censored on the end of AB period date otherwise. For cows that did not conceive (223,567), intervals to the conception were right-censored at the earlier of end of the mating date or 35 d before the last negative PD date. The models included milking regimen, breed, parity, interaction of milking regimen and breed, interaction of milking regimen and parity, linear and quadratic effects of the interval from calving date to mating start date as fixed effects and herd as frailty effect. The predicted probabilities of first service and conception by day from start of the mating period are presented using survival curves with 95% CI. The Cox proportional hazard models were modified to obtain the predicted probabilities of first service and conception by day of breeding period with milking regimen by keeping all other variables equal to the median of the population. To achieve this, the categorical variable of breed was transformed to continuous as the proportion of F, heterosis of F × J and parity number was fitted as a continuous variable. The models included milking regimen, proportion of F, heterosis of F × J, parity number and linear and quadratic effects of the interval from calving to mating start date.

RESULTS

Table 2 presents herd characteristics with average milk production and average F and J proportions by milking regimen. Compared with TAD milking herds, OAD milking herds had a shorter average mating length, but average intervals from calving to the start of mating were similar in both milking herds. Averages of milk, fat, protein, and lactose yields were lowest in cows milking OAD for the entire lactation whereas cows milked TAD had the highest averages for milk, fat, protein, and lactose yields. Herds milked OAD had higher proportions of J and lower proportions of F than herds milked TAD.

Milking Regimen

Least squares means with 95% CI of each fertility trait for milking regimen and parity classes are presented in Table 3. Effects of milking regimen, breed, and parity were significant ($P < 0.05$) for all fertility traits except the 6-wk submission (**SR42**) which was not significantly different among milking regimens ($P > 0.05$). In comparison to cows milked TAD, cows milked OAD had better reproductive performance as evidenced by higher ($P < 0.05$), SR21 (>4.7%), PR21 (>8.9%), PR42 (>7.3%), CR21 (>5.9%) and lower NIC (<3.8%). The odds ratios and 95% CI of fertility traits

for OAD, OAD-M, OAD-P, and OAD-E milking regimens are presented with reference to cows milked TAD in Table 4. Compared with TAD milking, the estimated odds ratios for SR21, PR21, PR42, PRFS, CR21, and CR42 were significantly higher in cows milked OAD and odds ratio for NIC was significantly lower in cows milked OAD than TAD.

The outcomes of Cox proportional hazard models are shown in Table 5. Milking regimen and parity were significantly ($P < 0.001$) associated with time to event variables of SMFS, SMCO, and FSCO. Estimated hazard ratios for SMFS, SMCO, and FSCO were significantly greater in OAD than TAD, OAD-M, OAD-P, OAD-E (Table 5). Predicted probabilities for first service and conception by day from start of the breeding season for cows milked OAD and TAD are shown in Figures 2 and 3, respectively. Cows milked OAD had slightly increased probabilities for first service by day of the breeding season than TAD (Figure 2) and predicted probabilities associated with conception during the breeding season were considerably increased in OAD than TAD (Figure 3).

Hazard ratios for SMFS were significantly different between TAD and OAD-P; however, no significant differences were observed in the hazard ratios of SMCO and FSCO between cows milked TAD and OAD-M, OAD-P or OAD-E (Table 5). These findings align with the outcomes of the binary trait analyses of SR21, PR21, and PR42. Submission by 3 wk was significantly higher in cows milked OAD-P compared with TAD. Pregnant by 3 wk and 6 wk was not significantly different between cows milked TAD and OAD-M, OAD-P, or OAD-E (Table 4). Cows milked OAD-M and OAD-P had significantly lower odds ratios for NIC compared with TAD.

Overall, cows milked OAD for the entire season had significantly better reproductive performance ($P < 0.05$) than cows milked OAD for part of the milking season (Tables 3–5). Cows milked OAD for part of the lactation are likely to have a small increase in reproductive performance compared with cows milked TAD.

Parity

Reproductive performance by parity is presented in Tables 3 and 5. Estimated hazard ratios for SMFS, SMCO, and FSCO were significantly different between first-parity cows compared with cows in second, third, fourth, and fifth or greater parity (Table 5). Overall reproductive performance as defined by SR21, PR42, and NIC was worst in the oldest (fifth or greater parity) and youngest (first parity). The general pattern for the binary traits (Table 3) was an improvement in fertility

from first to third parity, then a plateau in performance in the fourth parity with performance declining from the fifth parity onwards.

DISCUSSION

In seasonal dairy systems farmers aim to minimize the interval between the start of mating and conception. A key performance metric in seasonal systems is the proportion of the herd that are mated within 21 d of the start of the breeding season. Recent studies in other seasonal herds reported mean SR21 in Irish (74%, Coffey et al., 2016, and 76%, Kelleher et al., 2016) and Australian herds (67%, Morton et al., 2016), below the 79% (95% CI = 78.4–80.0) SR21 in our study. Each service needs to have a high probability of pregnancy in order for the herd to maintain a compact calving period. We report mean PRFS of 55% (95% CI = 54.2–55.8), higher than in year-round calving systems in the United States, Europe, and Great Britain (range from 37–52%; Royal et al., 2000; Mayne et al., 2002; Sørensen et al., 2008; Norman et al., 2009; Kelleher et al., 2016; O'Sullivan et al., 2020). We found New Zealand dairy cows had fewer SC (1.39 in 95% CI = 1.38–1.40) than cows in Ireland and the United Kingdom (1.5–1.8; Pryce et al., 1997; O'Sullivan et al., 2020). The relatively higher 3-wk submission and conception to first service result in PR42 of 68% (95% CI = 67.2–68.4), a higher mean PR42 than that of Australian (49%, Morton et al., 2016) and Irish dairy cows (58%, O'Sullivan et al., 2020) and a higher mean CR42 (83% in 95% CI = 82.2–83.5) than that of Irish dairy cows (66%, Coffey et al., 2016, and 70%, Kelleher et al., 2016).

In seasonal calving herds, fertility performance is commonly assessed using both PR42 and NIC (McDougall, 2006) and, PR42 is affected by submission rates and conception rates (Xu and Burton, 2003). Despite the relatively high reproductive performance compared with other countries, the cows in our study did not meet the New Zealand national reproduction targets of 78% PR42 and 8% NIC, although cows milked OAD were closer to the target (75% PR42 and 9% NIC) than cows milked TAD (68% PR42 and 13% NIC). However, our results demonstrate cows milked OAD achieved the industry targets of PRFS, CR21 and CR42.

There is a paucity of literature on the effect of OAD and TAD milking on cow fertility. Clark et al. (2006) reported mean SMCO was 3 d shorter in cows milked OAD than cows milked TAD. Our results support the findings of Clark et al. (2006) demonstrating that OAD milking cows are more likely to conceive earlier than TAD milking cows. We found comparatively higher mean percentages for SR21, PRFS, PR21, and PR42

Table 3. Least squares means (95% CI for means) of fertility traits for cows milking different parities under different milking regimens¹

Characteristic	Trait ²								
	SC	SR21	SR42	PR21	PR42	PRFS	NIC	CR21	CR42
Milking regimen ³									
OAD	1.31 ^b (1.29–1.33)	83.66 ^a (82.57–84.69)	92.47 ^a (91.68–93.18)	52.76 ^a (51.31–54.21)	75.08 ^a (73.93–76.21)	63.20 ^a (61.57–64.80)	8.90 ^c (8.26–9.58)	63.05 ^a (61.39–64.68)	87.48 ^a (86.35–88.53)
TAD	1.39 ^a (1.38–1.40)	78.96 ^c (78.36–79.55)	92.66 ^a (92.31–92.99)	43.86 ^b (43.18–44.54)	67.81 ^b (67.18–68.43)	55.00 ^b (54.19–55.81)	12.67 ^a (12.26–13.08)	57.12 ^b (56.30–57.93)	82.89 ^b (82.23–83.54)
OAD-M	1.40 ^a (1.36–1.43)	79.40 ^{bc} (77.24–81.40)	91.73 ^a (90.28–92.98)	43.55 ^b (41.60–45.53)	68.23 ^b (66.42–69.99)	53.97 ^b (51.60–56.33)	11.10 ^b (10.09–12.21)	56.51 ^b (54.02–58.96)	82.73 ^b (80.65–84.62)
OAD-P	1.38 ^a (1.36–1.39)	80.35 ^b (79.61–81.08)	93.02 ^a (92.58–93.44)	44.69 ^b (43.87–45.52)	68.81 ^b (68.07–69.55)	56.05 ^b (55.07–57.02)	11.29 ^b (10.85–11.75)	56.76 ^b (55.74–57.78)	83.20 ^b (82.38–83.98)
OAD-E	1.38 ^a (1.36–1.40)	79.80 ^{bc} (78.63–80.93)	92.86 ^a (92.18–93.49)	44.31 ^b (43.09–45.54)	68.39 ^b (67.28–69.49)	55.77 ^b (54.31–57.22)	12.47 ^a (11.77–13.21)	56.87 ^b (55.39–58.34)	83.44 ^b (82.25–84.55)
Parity									
1	1.39 ^a (1.38–1.40)	77.74 ^d (77.06–78.40)	91.20 ^c (90.78–91.60)	42.86 ^d (42.21–43.52)	66.98 ^d (66.35–67.59)	54.15 ^d (53.33–54.97)	12.26 ^b (11.87–12.67)	55.10 ^c (54.28–55.92)	81.38 ^c (80.68–82.06)
2	1.37 ^c (1.36–1.38)	79.87 ^b (79.24–80.49)	92.49 ^b (92.12–92.84)	46.36 ^c (45.68–47.05)	70.94 ^c (70.35–71.53)	57.26 ^c (56.43–58.09)	10.40 ^c (10.05–10.76)	57.50 ^b (56.68–58.33)	83.53 ^b (82.89–84.16)
3	1.36 ^d (1.35–1.37)	82.56 ^a (81.97–83.14)	93.83 ^a (93.52–94.13)	49.31 ^a (48.60–50.01)	73.42 ^a (72.84–74.00)	59.45 ^a (58.60–60.30)	9.33 ^d (8.99–9.67)	60.15 ^a (59.31–60.98)	85.64 ^a (85.04–86.22)
4	1.36 ^d (1.35–1.37)	82.90 ^a (82.30–83.49)	93.80 ^a (93.48–94.11)	48.26 ^b (47.54–48.98)	71.97 ^b (71.35–72.58)	58.54 ^b (57.65–59.42)	10.28 ^c (9.91–10.66)	59.86 ^a (58.99–60.72)	85.29 ^a (84.66–85.90)
≥5	1.38 ^b (1.37–1.39)	79.01 ^c (78.38–79.62)	91.02 ^d (90.59–91.42)	42.39 ^d (41.75–43.04)	64.95 ^e (64.33–65.56)	54.15 ^d (53.35–54.95)	14.35 ^a (13.92–14.80)	57.77 ^b (56.96–58.57)	84.07 ^b (83.46–84.67)
<i>P</i> -values for interaction ⁴									
Milking regimen × breed	0.72	0.36	0.27	0.61	0.72	0.42	0.18	0.28	0.06
Milking regimen × parity	0.98	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	0.14

^{a–e}Means with different superscripts, within the same column and factor, are significantly different ($P < 0.05$).

¹Least squares means estimates of binary fertility traits are represented as percentages. Models were fitted with fixed effects of milking regimen, parity, breed, and interval from calving to mating start date (linear and quadratic effects) as covariates; herd was fitted as a random effect.

²SC = number of services per conception; Binary (yes/no) traits; SR21 = cow inseminated in the first 3 wk from the start of mating; SR42 = cow inseminated in the first 6 wk from the start of mating; PR21 = cow conceived in the first 3 wk from the start of mating; PR42 = cow conceived in the first 6 wk from the start of mating; PRFS = cow conceived to first service; NIC = cow not in calf at end of the breeding season; CR21 = cow calved in the first 3 wk from the planned start of the calving; CR42 = cow calved in the first 6 wk from the planned start of the calving.

³OAD = full season once daily milking; TAD = full season twice daily milking; OAD-M = once daily milking in cow's mating period; OAD-P = once daily milking after peak lactation; OAD-E = once daily milking at end of the lactation.

⁴Interactions of milking regimen and breed were not significant for any fertility traits (Supplemental File S1, Figure S1.1–S1.9, <https://doi.org/10.7910/DVN/5HQOJD>), and interactions of milking regimen and parity were significant for all fertility traits except SC and CR42 (Supplemental File S2, Figure S2.1–S2.9, <https://doi.org/10.7910/DVN/5HQOJD>).

Table 4. Odds ratios (95% CI) and *P*-values of binary fertility traits for different milking regimens with reference to twice daily milking (TAD) category

Trait ²	Milking regimen ¹							
	OAD		OAD-M		OAD-P		OAD-E	
	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value	OR (95% CI)	<i>P</i> -value
SR21	1.36 (1.25–1.48)	<0.001	1.02 (0.90–1.17)	0.35	1.09 (1.03–1.16)	0.001	1.05 (0.97–1.14)	0.08
SR42	0.97 (0.86–1.09)	0.65	0.88 (0.73–1.06)	0.17	1.06 (0.97–1.15)	0.19	1.03 (0.92–1.15)	0.59
PR21	1.43 (1.34–1.52)	<0.001	0.99 (0.91–1.07)	0.77	1.03 (0.99–1.08)	0.12	1.02 (0.96–1.08)	0.52
PR42	1.43 (1.34–1.53)	<0.001	1.02 (0.94–1.11)	0.66	1.04 (0.99–1.09)	0.06	1.03 (0.97–1.09)	0.36
PRFS	1.41 (1.30–1.52)	<0.001	0.96 (0.87–1.06)	0.42	1.04 (0.99–1.10)	0.10	1.03 (0.97–1.10)	0.36
NIC	0.67 (0.62–0.74)	<0.001	0.86 (0.77–0.96)	0.009	0.88 (0.83–0.93)	<0.001	0.98 (0.91–1.06)	0.63
CR21	1.28 (1.19–1.38)	<0.001	0.98 (0.88–1.08)	0.64	0.99 (0.94–1.04)	0.59	0.99 (0.93–1.06)	0.77
CR42	1.44 (1.29–1.61)	<0.001	0.99 (0.86–1.14)	0.88	1.02 (0.95–1.10)	0.56	1.04 (0.95–1.14)	0.41

¹OAD = full season once daily milking; TAD = full season twice daily milking; OAD-M = once daily milking in cow's mating period; OAD-P = once daily milking after peak lactation; OAD-E = once daily milking at end of the lactation; OR = odds ratio. Models were fitted with fixed effects of milking regimen, parity, breed, and interval from calving to mating start date (linear and quadratic effects) as covariates; herd was fitted as a random effect.

²Binary (yes/no) traits: SR21 = cow inseminated in the first 3 wk from the start of mating; SR42 = cow inseminated in the first 6 wk from the start of mating; PR21 = cow conceived in the first 3 wk from the start of mating; PR42 = cow conceived in the first 6 wk from the start of mating; PRFS = cow conceived to first service; NIC = cow not in calf at end of the breeding season; CR21 = cow calved in the first 3 wk from the planned start of the calving; CR42 = cow calved in the first 6 wk from the planned start of the calving.

for cows milked OAD rather than TAD. Cows milked OAD were 43% more likely to be pregnant after 21 d and 42 d of breeding than TAD milking cows. Cows that conceive sooner after the start of mating calve earlier the next calving season and therefore have the opportunity for more days in milk before the final herd dry-off date for the season. Herds milked OAD had a higher proportion of cows calved by 3 wk and 6 wk, and therefore less late-calving cows. Because the start of mating is 83 d after the PSC the more compact calving pattern in OAD herds allows cows more time to recover from calving and resume estrus activity before the start of mating than cows milked TAD. This is likely to be one mechanism for the relatively better reproductive performance in cows milked OAD. Roche et al. (2007) reported a longer interval between calving and the start of mating and calving to first service positively affect the probability of successful conception to first service and pregnancy within the first 21 d from the start of mating. In our study OAD herds achieved a lower percentage of cows NIC despite a shorter average mating length (70 d) than TAD herds (76 d). Cows milked OAD were 33% less likely to be NIC than cows milked TAD (odds ratio = 0.67, 95% CI = 0.62–0.74, $P < 0.001$).

Our results show herds that milked OAD for the whole lactation had comparatively better fertility performance than herds that switched to OAD milking for part of the lactation. This provides further evidence that factors associated with OAD milking in early lactation led to improvements in reproductive performance. Furthermore, our results demonstrate submission by 3 wk, in calf by 3 wk, and in calf by 6 wk were not significantly different between the OAD-M and TAD milking systems. Rhodes et al. (1998) reported OAD milking of anoestrus cows from 7 d before the start of mating resulted in a higher proportion of cows detected in estrus compared with TAD milking, but pregnancy rates by 28 d after the start of mating were not significantly different in cows milked OAD or TAD. In contrast, we did find a small positive effect of milking OAD around mating on NIC. Cows milked OAD-M were 14% less likely to be NIC than cows milked TAD. This indicates that switching to OAD milking during the breeding season had a small positive effect on cow fertility in this study. It should be noted that we do not know when the farmers started OAD milking, relative to the start of mating and the reasons the farmers had used for switching to OAD were not quantified. It may be that herds experiencing poor fertility or challeng-

Table 5. Results of the Cox proportional hazard models for fertility traits in different milking regimens with reference to twice daily milking (TAD) and different parities with reference to parity 1

Characteristic	Trait ¹					
	SMFS		SMCO		FSCO	
	HR (95% CI)	<i>P</i> -value	HR (95% CI)	<i>P</i> -value	HR (95% CI)	<i>P</i> -value
Milking regimen ²						
OAD	1.05 (1.01–1.10)	0.01	1.23 (1.17–1.29)	<0.001	1.24 (1.18–1.30)	<0.001
OAD-M	0.97 (0.91–1.03)	0.34	0.99 (0.93–1.06)	0.83	0.98 (0.92–1.05)	0.61
OAD-P	1.03 (1.00–1.06)	0.05	1.03 (1.00–1.07)	0.09	1.03 (1.00–1.07)	0.08
OAD-E	1.01 (0.98–1.05)	0.47	0.99 (0.95–1.04)	0.69	1.00 (0.96–1.04)	0.92
Parity						
2	1.06 (1.05–1.07)	<0.001	1.10 (1.09–1.11)	<0.001	1.10 (1.09–1.11)	<0.001
3	1.10 (1.10–1.11)	<0.001	1.16 (1.15–1.17)	<0.001	1.15 (1.14–1.16)	<0.001
4	1.08 (1.07–1.09)	<0.001	1.11 (1.09–1.12)	<0.001	1.10 (1.09–1.11)	<0.001
≥5	0.91 (0.90–0.91)	<0.001	0.90 (0.89–0.91)	<0.001	0.92 (0.91–0.93)	<0.001
<i>P</i> -values for interaction ³						
Milking regimen × breed		0.08		0.09		0.31
Milking regimen × parity		<0.001		<0.001		<0.001

¹SMFS = start of mating to first service; SMCO = start of mating to conception; FSCO = first service to conception; HR = hazard ratio. Models were fitted with fixed effects of milking regimen, parity, breed, and interval from calving to mating start date (linear and quadratic effects) as covariates; herd was fitted as a shared frailty.

²OAD = full season once daily milking; OAD-M = once daily milking in cow's mating period; OAD-P = once daily milking after peak lactation; OAD-E = once daily milking at end of the lactation.

³Interactions of milking regimen and breed were not significant for all fertility traits, and interactions of milking regimen and parity were significant for all fertility traits (Supplemental File S3, Table S3, <https://doi.org/10.7910/DVN/5HQOJD>).

ing environmental conditions were more likely to use the OAD-M system. This is an area that needs further research.

The key reproductive metric of PR42 was not significantly different between cows milked OAD for part of the lactation (OAD-P and OAD-E) and TAD. However, SR21 and NIC were significantly different between cows milked OAD-P and TAD. There is no biological mechanism to explain the difference in SR21 between OAD-P and TAD herds, given that the change to OAD milking occurred either after the mating period was over, or at the end of the mating period. The differences indicate that there could have been management factors that differed between OAD-P and TAD herds that we were unable to account for in our study. One possibility is that OAD-P herds had the same milking regimen in previous years and the increased SR21 is a carryover effect from management in the previous lactation. Once daily milking results in cows with a higher BCS than cows milked TAD (Clark et al., 2006). Cows milked OAD-P would be expected to result with higher BCS in late lactation and into the subsequent lactation than cows milked TAD; and higher BCS is

associated with improved reproductive performance (Pryce et al., 2001). However, the present study did not attempt to investigate the influence of adopting OAD-P and OAD-E on reproductive performance in the subsequent calving year.

Our results agree with a previous study by Hemming et al. (2018), although the magnitude of the difference in PR42 between herds milked OAD and TAD was less in the current study (PR42 7.3% higher vs. 10.4% higher). The difference may be explained by the relative performance of the TAD herds. Reproductive performance in the TAD milking group in the current study (SR21 of 79% and PRFS of 55%) was greater than reported by Hemming et al. (2018) (SR21 of 77% and PRFS of 52%). National reproductive performance metrics in the 2017–2018 season are similar to reported SR21, PR42, and PRFS values in the TAD herds in the current study (DairyNZ, 2020). Edwards (2018) used calving dates, relative to the start of calving, as a proxy for reproductive performance. The proportion of cows calved by 3 wk and 6 wk after the start of calving was similar to that reported here. However, NIC percentages in the current study are lower than those reported by

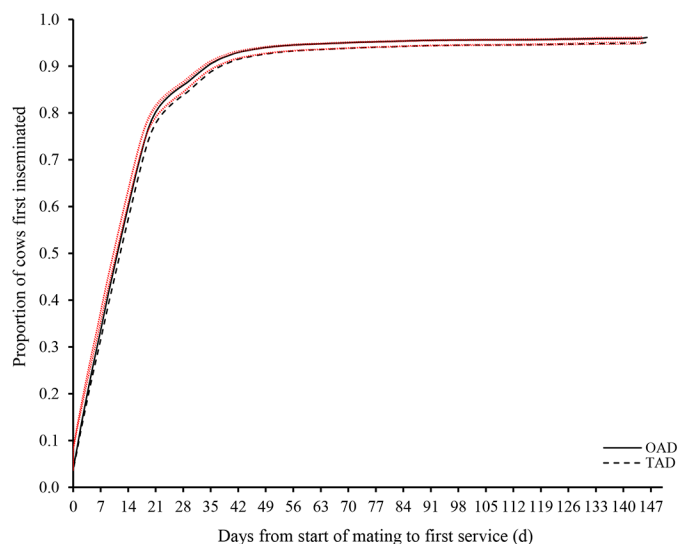


Figure 2. Predicted proportions of cows first inseminated by day from start of the mating period from the Cox proportional hazard model in cows milking under once daily (OAD, solid line) and twice daily (TAD, dashed line) with 95% CI (dotted lines). Predictions were adjusted for proportion of Holstein-Friesian, heterosis of Holstein-Friesian \times Jersey, parity number, linear and quadratic effects of interval from calving to mating start date and herd was fitted as a shared frailty.

Hemming et al. (2018), although the magnitude of the difference between OAD and TAD was similar. They reported OAD milking herds had a significantly lower percentage (12.8%) of cows NIC compared with TAD (17.6%) despite having a 4.3-d shorter mating period. Hemming et al. (2018) used the InCalf Fertility Focus User Guide (2015) definition of NIC, which is different to the classification of NIC in the present study, where cows without PD results and no herd test records after the end of the mating period were excluded from the calculation of NIC. The absolute values of NIC are, therefore, not comparable between the 2 studies, as the exclusion of cows without records may have underestimated the NIC value in our study.

In New Zealand, most dairy cows are artificially inseminated in the first few weeks of the mating season and then bulls are run with the herd until the end of the breeding season. In general, bull services are poorly recorded and dates where the bulls were removed from herds were not available and could only be estimated based on the conception dates. The herds included in present study had both AB and natural services records and this is one limitation of this study.

Results of the present study show that cows milked OAD for the whole lactation are more fertile than cows milked TAD. One potential mechanism for the difference in fertility performance between cows milked OAD or TAD is reduced NEB in early lactation (Phyn et al.,

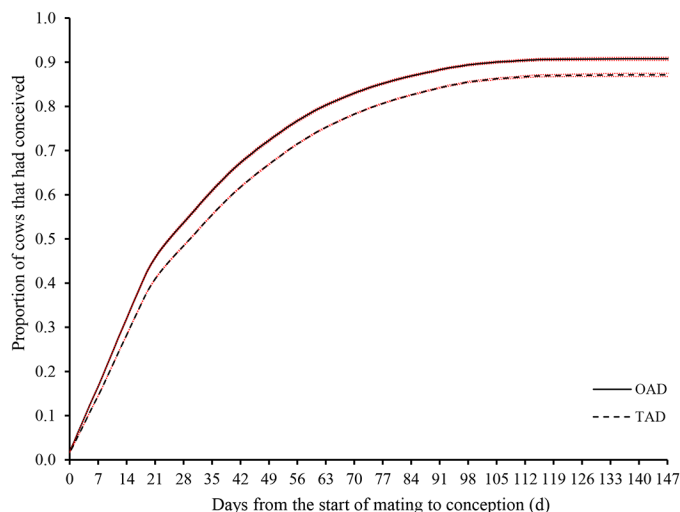


Figure 3. Predicted proportions of cows that had conceived by day from start of the mating period from the Cox proportional hazard model in cows milking under once daily (OAD, solid line) and twice daily (TAD, dashed line) with 95% CI (dotted lines). Predictions were adjusted for proportion of Holstein Friesian, heterosis of Holstein Friesian \times Jersey, parity number, linear and quadratic effects of interval from calving to mating start date and herd was fitted as a shared frailty.

2010; Kay et al., 2013). Phyn et al. (2014) found that cows milked OAD after calving have greater plasma glucose and lower plasma nonesterified fatty acid concentrations than cows milked TAD. Butler (2000) reported NEB during the first 3 or 4 wk of lactation, is highly correlated with the interval to first ovulation and delays the conception of cows. Clark et al. (2006) and Phyn et al. (2014) reported that cows milked OAD had an improved BCS compared with cows milked TAD. Body condition score has been correlated with reproductive performance. Roche et al. (2007) found postcalving BCS is positively associated with cycling by the start of mating, SR21, PRFS, PR21, and PR42. Clark et al. (2006) reported higher SR21 and pregnancy rate in cows milked OAD and improved BCS after calving than those milked TAD.

Investigations of the effect of OAD milking on milk production in New Zealand clearly demonstrate that herds milked OAD produce less milk than herds that milk TAD (Phyn et al., 2010; Stelwagen et al., 2013; Lembeye et al., 2016; Edwards, 2018). In agreement with the previously cited literature, cows milked OAD for entire lactation produced lower milk, fat, protein and lactose yields than the cows milking in TAD, OAD-M, OAD-P, and OAD-E herds. Although this study cannot establish a causal relationship between milking frequency and reproductive performance the existing literature establishes potential mechanisms whereby the reduction in yield caused by OAD milking could

positively influence reproductive performance via effects on BCS and NEB.

In New Zealand, approximately 40% of the total annual industry costs were associated with cow wastage that were recorded as culled due to failure to conceive (31%) and low production (8%; Kerslake et al., 2018). Thus, OAD milking herds may have a reduced cow wastage from fewer nonpregnant cows and reduced replacement costs. The superior reproductive performance of OAD herds provides an opportunity for these herds to either lower replacement rates or remove a higher proportion of low production, or otherwise less suitable cows. Edwards (2018) reported cows culled from herds milking OAD for the entire season were less likely to be culled for failure to conceive and more likely to be culled due to low production, udder health and mastitis. Replacement of the low production cows allows genetically superior animals to enter the herd, increasing rates of genetic gain.

Consistent with previous investigations, fertility also differed with parity (Xu and Burton, 2003). Poorer reproductive performance was observed in the youngest and oldest animals within a herd, consistent with the findings of Roche et al. (2007). First-parity cows had the lowest SR21, indicative of extended postpartum anoestrus intervals in this age group (McDougall, 2006). Cows of parity ≥ 5 had the lowest PR42 and highest NIC, with nearly 20% of this group failing to conceive. Herds with high proportions of older cows are at risk of poorer reproductive performance, a factor to consider when determining the optimum herd replacement rate. Although the interactions between milking regimen and parity were statistically significant for all studied fertility traits except SC and CR42, the size of the effects were small.

CONCLUSIONS

Cows milked OAD for the entire lactation were more fertile than cows milked TAD for the entire lactation. There was no significant difference in the key reproductive metric of PR42 between herds milked OAD for part of the lactation or herds milked TAD, but NIC was significantly different between herds milked OAD-M, OAD-P, and TAD. These findings indicate that most of the reproductive benefits of OAD milking are related to OAD milking in early lactation, most likely via changes to milk production, BCS, and reduced NEB before mating. Fertility outcomes were significantly different between different parities. Cows in their third and fourth parities had superior reproductive performance than first-parity cows, whereas older cows (parity ≥ 5) had the poorest reproductive outcomes. The present study indicates the adopting OAD milking for the en-

tire lactation was associated with improved reproductive outcomes.

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