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Genetic parameters of fertility traits of cows milked once daily versus twice daily in New Zealand

A thesis presented in partial fulfilment of the requirements for

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Abstract

In New Zealand about 55% of dairy herds are milked twice daily (TAD) and about 9% of herds are milked once daily (OAD) for their entire lactation, with the balance of herds using variable milking frequencies across the lactation. The general aim of this thesis was to estimate genetic parameters for fertility traits of spring-calved OAD and TAD milking cows in New Zealand.

Cows milked OAD for the entire lactation were more fertile than cows milked TAD for the entire lactation. Heritability and repeatability estimates for milk production and fertility traits were broadly consistent for cows that were milked either OAD or TAD for their entire lactation. Though genetic correlations between milk production and fertility were antagonistic in both milking systems, correlations between milk production and the key reproductive metrics of 6 wk in-calf, not in-calf rate and 6 wk calving were less strong in cows milked OAD compared with TAD.

In both OAD and TAD milking populations, individual breed effects for milk, fat, protein and lactose yields were in favor of Friesian (F) cows; however, maternal breed effects for yields were in favor of Jersey (J) dams. Jersey cows were more fertile than the F cows in both milking populations, but maternal breed effects for fertility traits were in favour of F dams. Favourable F×J heterosis for milk production and fertility traits were significant in both milking cow populations. Recombination loss effects were sometimes favorable for production and fertility traits, but most estimates were small with larger standard errors.

A genome-wide association study (GWAS) was conducted using 1,537 spring-calved dairy cows genotyped with Bovine Illumina 50K single nucleotide polymorphisms

(SNPs) chips in two Massey University dairy herds. Four SNPs on chromosome 14 were highly significant for fat and protein percentages and associated genes were DGAT1, SLC52A2, CPSF1 and MROH1. The GWAS identified 10 candidate genes (KCNH5, HS6ST3, GLS, ENSBTAG00000051479, STAT1, STAT4, GPD2, SH3PXD2A, EVA1C and ARMH3) associated with fertility traits, which relate to functions, reduced metabolic stress and increased insulin secretion during the mating period, early embryonic development, fetal growth, and maternal lipid metabolism during the pregnancy period.

Overall, the results of this thesis indicate milking OAD for the entire lactation improves the fertility performance of dairy cows but reduces milk production. The antagonistic genetic correlations between milk yield and key fertility measures were weaker in the OAD milking population than in the TAD milking population. The findings of this thesis can be used to develop a selection index for production and fertility traits in OAD milking cow population in New Zealand.

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List of Abbreviations

AB	Artificial breeding
AC	Age at calving
BCS	Body condition score
BW	Breeding worth
CFS	Calving to first service
CI	Calving interval
CR21	3 weeks calving rate
CR42	6 weeks calving rate
DIM	Days in milk
F	Holstein-Friesian
FP	Fat percentage
FSCO	First service to conception
FY	Fat yield
GWAS	Genome-wide association studies
J	Jersey
LP	Lactose percentage
LY	Lactose yield
MY	Milk yield
NEB	Negative energy balance
NIC	Not in-calf
OAD	Once daily
PD	Pregnancy diagnosis
PP	Protein percentage
PR21	3 weeks in-calf rate
PR42	6 weeks in-calf rate
PRFS	Pregnant to first service
PSC	Planned start of calving
PSM	Planned start of mating
PW	Production worth
PY	Protein yield

SC	Services per conception
SMCO	Start of mating to conception
SMFS	Start of mating to first service
SNP	Single nucleotide polymorphism
SR21	3 weeks submission rate
SR42	6 weeks submission rare
TAD	Twice daily

Chapter 1

General Introduction

Good reproductive performance is a key driver of farm profitability in New Zealand’s pasture-based dairy systems. Poor fertility performance of dairy cows leads to reduced milk production per cow during the lactation, longer calving periods and increased replacement costs and rates of involuntary culling. Kerslake et al. (2018) reported that reproductive failure is the primary cause of cow wastage in New Zealand dairy herds, where about 31% of the total annual industry cost is associated with cows that were recorded as culled due to non-pregnancy. The New Zealand dairy industry has targets for herd-level fertility measures of 3-week submission rate (SR21; 90%), pregnant to the first service (PRFS; 60%), 6-week in-calf rate (PR42; 78%), not-in-calf rate at the end of mating season (NIC; 8%), and 6-week calving rate (CR42; 87%) (Burke and Fowler, 2007). However, national averages for fertility are below these targets. There is a significant variation in herd-level reproductive performance, even in herds with similar genetics. Xu and Burton (2003) reported that PR42 had the highest variation in herd-level performance; the top quartile herds achieved 78% PR42 and the bottom quartile 57% in New Zealand. The average performance of the top quartile herds in the study of Xu and Burton (2003) was used to the set the PR42 target.

In New Zealand, the genetic evaluation of dairy cow fertility is currently performed using SR21, CR42 and calving season day (Amer et al., 2016). Previous studies have reported that the heritabilities for these traits ranged from 0.015 to 0.058 in New Zealand dairy cattle, are generally low (Harris et al., 2005; Bowley et al., 2015; Amer et al., 2016). Thus, management and environmental factors have a greater influence on dairy cow fertility than genetic factors.

Since 1990, some dairy farmers in New Zealand have adopted once daily (OAD) milking (Davis et al., 1999) and in the production season of 2015-2016, 9% of the tested herds practiced OAD milking in the entire season (Edwards, 2018). The distribution of OAD herds varies across the country, 31% of dairy farmers in Northland practiced full season OAD milking (Dairy NZ, 2023). Previous studies have demonstrated that herds milking OAD for the entire season had improved reproductive performances compared with the twice daily (TAD) milking herds for the entire lactation in New Zealand (Edwards, 2018; Hemming et al., 2018). However, genetic parameters for fertility traits have not been estimated in the OAD milking cow population.

At present the New Zealand national herd is comprised of 59.2% of Holstein-Friesian×Jersey cows (F × J), followed by 25.2% of Holstein-Friesian (F) and 7.6% of Jersey (J) cows (LIC and DairyNZ, 2022). Previous studies have reported the breed and heterosis effects for milk production traits in OAD milking cow population (Lembeye et al., 2016b; Lembeye et al., 2016c), however, recombination loss effects for milk production and fertility traits have not been reported. Maternal breed and heterosis effects for milk production have been reported in New Zealand dairy cows (Ahlborn-Breier and Hohenboken, 1991), but the maternal breed, heterosis and recombination loss effects for fertility traits are unknown. Due to the larger proportion of crossbred animals in the national herd, and the number of OAD milking herds, it is important to investigate if the heterosis effects on cow fertility differ across the milking frequency populations.

Antagonistic genetic correlations have been reported between milk production and fertility traits in New Zealand dairy cows (Grosshans et al., 1997; Harris et al., 2006).

The recent development of genomic selection on fertility traits has stabilized and even reversed the decreasing trend of cow fertility (Ma et al., 2019). Since 2008, genomically enhanced breeding values have been used for the genomic selection in New Zealand dairy cattle (Harris et al., 2008; Winkelman et al., 2015). No published GWAS were found in the literature reporting candidate genes associated with key reproductive metrics of SR21, PR42, CR42 and NIC either in New Zealand or overseas.

Heritability and repeatability estimates for milk production traits and genetic and phenotypic correlations between milk yields and milk composition traits have been reported with OAD milking cow population in New Zealand (Lembeye et al., 2016a). Furthermore, Lembeye et al. (2021) reported that genetic correlation estimates for same milk production traits expressed in the two milking frequencies (OAD and TAD) were lower than unity. The genetic and phenotypic correlations between milk production and fertility traits could be different across the milking frequency population because the extent of negative energy balance in early lactation is reduced for cows milked OAD compared with the cows milked TAD (Phyn et al., 2010; Kay et al., 2013).

The aim of this thesis was to estimate heritability and repeatability for fertility traits and genetic and phenotypic correlations for milk production and fertility traits in spring calved OAD and TAD milking cows in New Zealand. The specific objectives proposed to achieve this main aim were:

1. To investigate fertility performance of cows milked OAD and TAD.

2. To estimate heritability, repeatability and individual and maternal breed, heterosis and recombination loss effects for milk production and fertility traits of seasonally calved dairy cows milked OAD and TAD.
3. To estimate genetic correlations for milk production and fertility traits in cows milked OAD and TAD.
4. To conduct a genome-wide association study for milk composition and fertility traits.

The findings of this thesis will add to knowledge of genetic parameters including variance components, heritability, repeatability, genetic and phenotypic correlations linked with milk production and fertility traits of spring-calved cows milked OAD in New Zealand dairy herds. Furthermore, the results of GWAS for fertility traits will provide a foundation for future research studies on genomic selection on dairy cow fertility in New Zealand.

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Chapter 2

Review of Literature

A comprehensive review of literature discussing milk production and fertility performance of spring-calved dairy cattle milked either OAD or TAD during the entire lactation was presented. This review examined the breed, heterosis and recombination loss effects for milk production and fertility traits in different dairy cattle populations. Moreover, genetic parameters for milk production and fertility traits, genomic regions and candidate genes associated with milk composition and fertility traits of New Zealand dairy cows were also reviewed.

2.1 New Zealand milk production system

The seasonally concentrated calving pattern under pastoral management is a unique feature of New Zealand dairy farming. The majority (95%) of New Zealand dairy farms calve during winter (Blackwell et al., 2010). Calving is timed to match the feed requirement of the dairy cows with the pasture growth (Verkerk, 2003). The breeding season starts at a fixed date in Spring (between late September and early November) and every cow detected in oestrus in the herd is inseminated regardless of the number of days since calving (Grosshans et al., 1997). 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 a concentrated calving pattern in the next season (Xu et al., 1997)

To maintain the same calving date from year-to-year cows need to achieve a 365 day inter-calving interval. To achieve this requires good reproduction management practices (Macmillan and Curnow, 1976; Holmes, 2001). The study of Macmillan and Curnow (1976) documented that the average gestation length in New Zealand dairy cows was 282 ± 4.5 days (± S.D.) with a normal gestation range of 272 to 293 days, which leaves a maximum of 83 days for the cow to resume ovarian cycling, be mated and conceive (Blackwell et al., 2010). Therefore, one of the key requirements of reproductive performance in the seasonal dairy production system is the cow initiating ovarian cyclicity soon after calving and to be mated and conceived within 6 weeks after the start of the breeding season (Grosshans et al., 1997).

Since 1985, crossbreeding has been adopted as the predominant mating strategy in New Zealand (Montgomerie, 2005). At present New Zealand national herd is comprised of crossbred Holstein Friesian×Jersey (F×J; 59.2%) followed by Holstein

Friesian (F; 25.2%), Jersey (J; 7.6%), other breeds and crosses (7.6%), and a small proportion of Ayrshire (0.4%; LIC and DairyNZ, 2022).

2.2 Fertility measures in New Zealand dairy cows

The fertility measures used in the seasonal dairy production systems are different to those used in non-seasonal dairy systems. In New Zealand, many reproductive measures are derived considering the start of mating as a reference point, since the primary objective of New Zealand seasonal breeding system is for each cow to conceive as soon as possible after the date selected for the start of breeding (Grosshans et al., 1997). Macmillan (2002) reported that careful consideration for selecting the start date for the planned start mating was necessary for the occurrence of peak calving at the commencement of the next production season, from late winter to early spring.

The InCalf Fertility Focus User's Guide described the criteria for defining the start of the mating and end of the mating dates in New Zealand dairy herds (DairyNZ, 2019). The mating start date is defined as the first of 2 consecutive days with at least one artificial breeding (AB) insemination or natural service, where at least 3 of the next 6 days also had mating records (DairyNZ, 2019). The mating end date of the herd is defined as either the last recorded mating date on or before the 21st week of the mating period or the last date with 2 conceptions followed by at least 30 days with no conceptions on or before the 21st weeks of the mating period or the last date with one conception on or before the 21st weeks of the mating period followed by at least 30 days with no conceptions and had at least one conception on each of 2 or more other days in the 6 preceding days (DairyNZ, 2019).

There are several reproductive measures used by New Zealand farmers and scientists to measure the reproductive success of the herd. Fertility traits can be categorised into three types, interval traits, binary traits and count traits (Berry et al., 2014). In general, interval traits and binary counts are used to calculate the fertility of cows in New Zealand dairy herds (Grosshans et al., 1997; Harris et al., 2006; Brownlie et al., 2014). A graphical summary of the commonly described parameters has been shown in Figure 2.1.

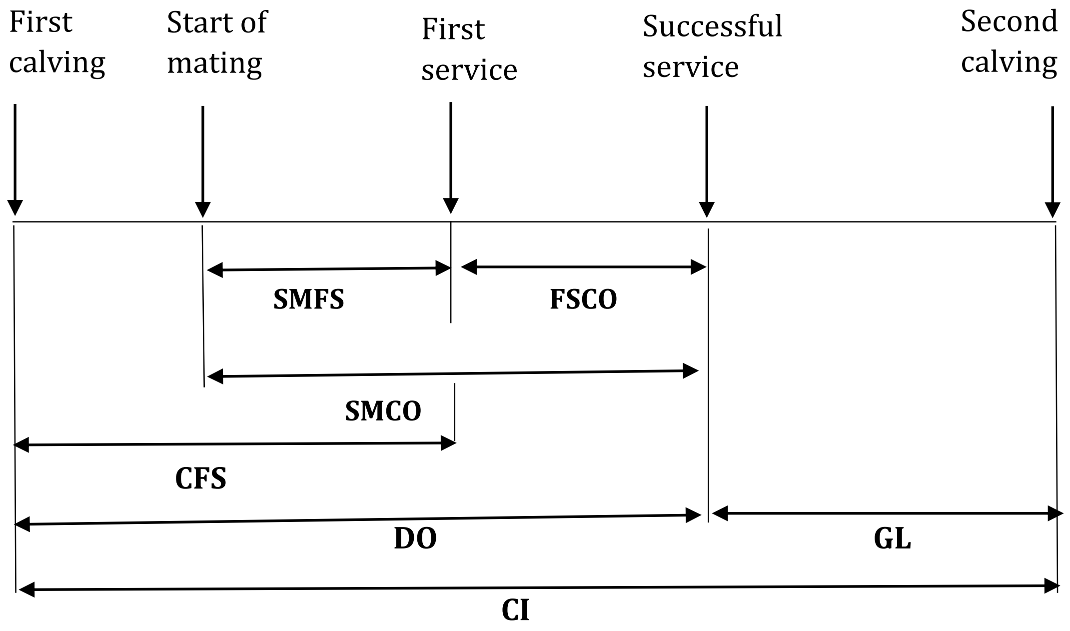

Figure 2.1. Graphical representation of the reproductive traits used to measure fertility performances of dairy cows in New Zealand dairy system. SMFS = interval from start of mating to first service, SMCO = interval from start of mating to conception, CFS = interval from calving to first service, FSCO = interval from first service to conception, DO = interval from calving to conception (days open), CI = interval between consecutive calvings, GL = gestation length. Adaptded from Grosshans et al. (1997).

2.2.1 Three-week submission rate

This measure describes the percentage of cows that received at least one artificial insemination or natural service in the first three weeks of the mating period, with respect to all cows of the herd that will be serviced (Brownlie et al., 2014). The average SR21 for herds in the top quartile was 91%, compared to 67% for herds in the bottom quartile (Xu and Burton, 2003).

2.2.2 Pregnancy rate to first service

Pregnancy rate to first service reflects the percentage of cows conceiving in a herd after their first insemination (Macmillan, 1974). The target conception rate for lactating New Zealand cattle is 60% (Xu and Burton, 1996). When comparing the conception rate of modern dairy cows with previously studied survey data, it is evident that in the PRFS of dairy cows has declined in New Zealand. Macmillan et al. (1984) indicated an average of 67.7% of PRFS for the season of 1982-1983 but, it had declined to 55% by 2006 (McNaughton et al., 2006), and it gradually increased from 50.1% to 52.7% during the season from 2008-2009 to 2018-2019 (LIC and DairyNZ, 2021).

2.2.3 Six-week in-calf rate

In seasonal calving herds, fertility performance is commonly assessed using both 6 weeks in-calf and not in-calf rates (McDougall, 2006). The proportion of cows that conceive in the first 6 weeks of the mating season (PR42; 6-week in-calf rate) is an outcome of both SR21 and PRFS (Xu and Burton, 2003). Hemming et al. (2018) reported that a reliable estimate for PR42 can be obtained from the results of early-aged pregnancy testing data (tested on or between 35 and 122 days of pregnancy).

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 a 78% PR42, and the bottom quartile 57%, indicative of a large effect of farm management on reproductive outcomes in New Zealand dairy herds.

2.2.4 Not in-calf rate

Not in-calf rate defines the percentage of the cows that were present at the start of mating, but that were not pregnant at the end of the mating period. This is a key reproductive indicator as cows that are not pregnant are typically removed from the dairy herd and will need to be replaced. 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 pregnant (Kerslake et al., 2018). Also, NIC is relative to the length of the herd's mating period. This means comparing NIC between herds can be problematic. An analysis of New Zealand national herd reported NIC as 17% for an average mating length of 75 days in production season 2017 (LIC, 2023).

2.2.5 Six-week calving rate

Six-week calving rate measures the proportion of cows that were calved within the first 42 days of the planned start of calving date in the herd. The planned start of calving date is calculated for a herd as the mating start date in the previous season plus 282 days (DairyNZ, 2019). This is one of the key reproductive metrics included in the national breeding goal in New Zealand (Bowley et al., 2015).

A description of the most commonly reported fertility measures in literature with the target values of New Zealand dairy industry and national averages in the production year 2020-2021 is shown in Table 2.1.

Table 2.1. Most reported fertility measures in New Zealand studies

Trait[1]	Criteria for trait calculation	Target[2]	National average[3]
Individual cow measures			
SMFS	The interval from the start of mating to the first service (days)		
SMCO	The interval from the start of mating to conception (days)		
FSCO	The interval from the first service to conception (days)		
CFS	The interval from calving to the first service (days)		
DO	The interval from calving to conception (days)		
CI	Interval between the consecutive calving (days)	365 d	369 d
SC	Number of services for conception (count)		
CSD	Calving season day		
Herd measures			
SR21	Percentage of cows with the first mating date in the first 21 days from the start of mating date	90%	81.3%
SR42	Percentage of cows with the first mating date in the first 42 days from the start of mating date		
PR21	Percentage of cows conceived in the first 21 days from the start of mating date		
PR42	Percentage of cows conceived in the first 42 days from the start of mating date	78%	67.7%
PRFS	Percentage of cows conceived to the first AB insemination	60%	52.7%
NIC	Percentage of not in-calf at the end of the mating period	8%	16.8%
CR21	Percentage of cows calved in the first 21 days from planned start of calving date	60%	
CR42	Percentage of cows calved in the first 42 days from the planned start of calving date	87%	

[1] Source: Grosshans et al. (1997), Brownlie et al. (2014), Bowley et al. (2015), Edwards (2018).

[2] Source: Burke and Fowler (2007).

[3] Source: LIC and DairyNZ (2021).

Fertility performances of New Zealand and other dairy-producing countries are summarized in Table 2.2. Many overseas studies (Butler, 1998; Roche et al., 2000; Royal et al., 2000; Lucy, 2001) and New Zealand studies (Grosshans et al., 1997; Burke and Fowler, 2007) highlighted that the fertility performance of dairy cows declined in parallel with increasing milk production in past decades.

Table 2.2. Mean values for fertility traits[1] of dairy cows milked in New Zealand and other dairy producing countries

Reference	Country[2]	Breed[3]	Fertility trait[1]												
			SMFS (days)	SMCO (days)	FSCO (days)	CFS (days)	DO (days)	CI (days)	SC	SR21 (%)	PRFS (%)	PR21 (%)	PR42 (%)	NIC (%)	CR42 (%)
Macmillan et al. (1987)	NZ	-	12.9	24.8	-	77.2	88.3	-	-	84.0	59.0	54.6	-	5.1	-
Grosshans et al. (1997)	NZ	F	18.2	33.3	14.5	86.6	101.1	376	1.49	-	-	46.3	72.7	-	-
		J	15.2	29.9	14.7	81.7	96.4	373	1.51	-	-	52.0	77.4	-	-
Xu and Burton (2003)	NZ	F	-	-	-	-	-	-	-	83.0	56.0	-	70.0	-	-
		J	-	-	-	-	-	-	-	87.0	53.0	-	70.0	-	-
		F×J	-	-	-	-	-	-	-	87.0	56.0	-	72.0	-	-
Brownlie et al. (2014)	NZ	F, J, F×J	-	-	-	-	-	-	-	81.8	48.4	-	67.0	-	-
Mayne et al. (2002)	Ireland	F	-	-	-	83.7	-	407	-	-	37.1	-	-	-	-
Berry et al. (2003)	Ireland	HUK	-	-	16.7	72.8	-	-	-	1.80	49.0	-	-	-	-
Coffey et al. (2016)	Ireland		-	-	-	75.0	-	379	-	74.0	-	-	-	-	66.0
Kelleher et al. (2016)	Ireland	H,F,J,M	-	-	-	76.0	-	369	1.56	76.0	52.0	-	69.0	-	70.0
O'Sullivan et al. (2020)	Ireland	F	13.8	25.3	-	-	-	-	1.57	88.1	45.5	35.7	57.5	-	-
Morton et al. (2018)	Australia	-	-	-	-	83.6	112.8	391	-	66.1	38.0		47.1	20.8	-
Pryce et al. (1997)	UK	F	-	-	-	84.0	101.0	387	1.50	-	66.0	-	-	-	-
Royal et al. (2000)	UK	BF	-	-	-	-	-	-	-	-	55.6	-	-	-	-
		F	-	-	-	72.3	-	382	-	-	39.6	-	-	-	-

Table 2.2. Mean values for fertility traits¹ of dairy cows milked in New Zealand and other dairy producing countries (continued)

			Fertility traits²												
Reference	Country	Breed¹	SMFS (days)	SMCO (days)	FSCO (days)	CFS (days)	DO (days)	CI (days)	SC	SR21 (%)	PRFS (%)	PR21 (%)	PR42 (%)	NIC (%)	CR42 (%)
Hoekstra et al. (1994)	Netherlands	F	-	-	22.0	84.0	-	386	-	-	-	-	-	-	-
Norman et al. (2009)	US	H	-	-	-	86.0	144.0	422	2.50	-	31.0	-	-	-	-
		J	-	-	-	84.0	129.0	410	2.30	-	39.0	-	-	-	-
Sørensen et al. (2008)	Denmark	F, DR, FA	-	-	48.2	72.5	-	-	2.07	-	45.7	-	-	-	-
González-Recio et al. (2004)	Spain	H	-	-	-	81.0	117.0	400	1.87	-	48.0	-	-	-	-
Tiezzi et al. (2012)	Italy	BS	-	-	38.3	90.7	129.0	-	1.75	-	55.0	-	-	-	-
Pimentel et al. (2011)	Germany	-	-	-	99.5	98.7	99.0	-	-	-	-	-	-	-	-

[1] SMFS = start of mating to first service, SMCO = start of mating to conception, FSCO = first service to conception, CFS = calving to first service, DO = calving to conception/days open, CI = consecutive calving interval, SC = the number of services for conception, SR21 = cow inseminated in the first 3 wk from the start of mating, PRFS = cow conceived to first service, 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, NIC = cow not in-calf at end of the breeding season, CR42 = cow calved in the first 6 wk from the planned start of the calving.

[2] NZ = New Zealand, UK = United Kingdom, US = United States. [3] F = Holstein-Friesian, J = Jersey, F×J = crossbred Holstein-Friesian×Jersey, HUK = Holstein United Kingdom, H = Holstein, M = Montbéliarde, BF = British Friesian, BS = Brown Swiss, DR = Danish Red and FA = Finnish Ayrshire.

In general, it is evident that New Zealand dairy cattle had better fertility performance compared to Irish, Australian, Netherland, United States, Europe and Great Britain dairy cows (Table 2.2). However, national averages on SR21, PRFS, PR42 and NIC are below the New Zealand industry targets (Table 2.1).

2.3 Parity differences on fertility in New Zealand dairy cows

Parity or age effects on reproductive performance in lactating cattle have been reported in New Zealand. Grosshans et al. (1997) reported that second-parity cows had better reproductive performance than first-parity cows and had shorter intervals from the start of mating to first service (by -0.5 days), the start of mating to conception (by -0.9 days), first service to conception (by -0.8 days), calving to first service (by -9.8 days), calving to conception (by -10.6 days) and less number of services for conception (by 0.01) and higher PR21 (by 1.5 %) and PR42 (by 1.8%). Xu and Burton (2003) also reported that age and parity affected reproductive performance. Poorer reproductive performance with lower SR21, PRFS, PR42 and higher NIC rate 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).

There is little information available in the current literature on the fertility performance of New Zealand dairy cattle by parity. Also, information on fertility measures that are related to the intervals to insemination or conception from the start of the breeding season is scarce. Therefore, it is timely to investigate the fertility performances of New Zealand dairy cattle by parity and obtain an overview of present trends subject to the large population of commercial dairy herds in New Zealand.

2.4 Once daily milking system in New Zealand

Twice daily milking is accepted as the traditional milk removal frequency in New Zealand. Since the late 1990s, some farmers in New Zealand have been milking their cows OAD for the entire lactation (Davis et al., 1999), and about 9% of herds that underwent herd-testing in the production season 2015-2016 were milked OAD for the entire lactation (Edwards, 2018). Farmers cite several benefits from changing to OAD milking in New Zealand. These include ease of attracting labor, reduced labor costs, the opportunity for off-farm employment, management of feed shortfalls, herd expansion, allowing time to build capital, utilizing farmland with hilly terrain or long walking distances, herd health, and management (Bewsell et al., 2008).

2.5 Milk production of full-lactation OAD milking cows in New Zealand

In New Zealand, there have been four experimental studies that compared cows milked OAD and TAD during the entire lactation (Holmes et al., 1992; Cooper and Clark, 2001; Clark et al., 2006; Dalley et al., 2008). A study by Lembeye et al. (2016a) investigated the milk production of OAD and TAD milking cows using data from commercial herds in New Zealand. These studies are summarized in Table 2.3.

According to previous studies reducing milking frequency from TAD to OAD comes with a loss of milk production. Holstein Friesian cows milked OAD, yielded between 27.3% and 31.2% less milk per cow and less (24.3-29.5%) milksolids (fat + protein) per cow than TAD. Jersey cows were less affected than F cows by OAD milking with a reduction in milk yields per cow (18.9-22.1%) and milksolids per cow (13.8-20.1%). Crossbred Holstein-Friesian and Jersey cows yielded between 24.3% and 31.5% less milk yields and milksolids (19.6% to 30.0%) per cow milked OAD relative to their TAD counterparts.

Table 2.3. Mean values for lactation yields of milk, fat, protein, lactose and milk solids (kilograms of fat and protein) from dairy cows milked once daily (OAD) or twice daily (TAD) during the entire lactation in New Zealand

Study	Years	Breed[1]	Milking Frequency	Days in milk	Milk (kg/cow)	Fat (kg/cow)	Protein (kg/cow)	Lactose (kg/cow)	Milk solids (kg/cow)	Milk solids (kg/ha)
Holmes et al. (1992)	1990		TAD	253	4,320	208	162	217	370	-
			OAD	251	2,810	144	110	136	254	-
Cooper and Clark (2001)	1999	F×J	TAD	269	3,543	177	131	171	307	922
			OAD	255	2,427	122	93	115	215	753
Clark et al. (2006)	2000-2004	F	TAD	244	4,234	187	149	205	336	1,051
			OAD	230	2,914	131	106	138	237	879
		J	TAD	242	2,839	162	116	141	278	1,045
			OAD	229	2,211	129	94	107	222	979
Dalley et al. (2008)	2004-2006	J	TAD/OAD	274	3,196	189	136	-	325	1,277
			OAD	269	2,592	163	117	-	280	1,219
Lembeye et al. (2016a)	2008-2012	F	TAD	-	4,503	196	162	-	358	-
			OAD	-	3,275	149	122	-	271	-
		F×J	TAD	-	3,973	195	152	-	347	-
			OAD	-	3,009	157	122	-	279	-
		J	TAD	-	3,234	186	134	-	320	-
			OAD	-	2,575	152	111	-	263	-

[1] F = Holstein-Friesian, J = Jersey and F×J = crossbred Holstein-Friesian×Jersey.

2.6 Fertility performances of full-lactation OAD milking cows in New Zealand

There is a limited number of studies that reported the effect of OAD milking on the reproductive performance of cows in New Zealand (Table 2.4). Clark et al. (2006) reported that cows milked OAD conceived 3 days earlier than the counterpart of TAD whereas OAD milked cows required a smaller number of days (5) from calving to conception. Further, results showed cows milked OAD had significantly higher 3-week submission (by 7.3%) and a 3-week pregnancy (by 7.8%) than TAD milked cows. However, there was no significant effect of the milking frequency with a 6-week submission, 3-week conception, 6-week conception, days to the first oestrus, or the total number of inseminations per cow. The limitation of study by Clark et al. (2006) was the use of a small number of cows (65 total), therefore it did not have the statistical power to determine if OAD milking had any effect on reproductive performance.

Edwards (2018), reported OAD milked cows had significantly higher 3-week and 6-week calving rates compared to TAD milking cows (by 5%). Furthermore, finding of the study concluded that cows milked OAD were less likely to be culled due to not being pregnant. Nevertheless, the study of Edwards (2018), did not comprehensively study herd characteristics for measuring cows fertility other than calving rates.

A recent study by Hemming et al. (2018) compared a large number of herds milked OAD (156) and TAD (143) in whole seasons (2014 to 2016). They reported that cows milked OAD for the whole season had on average better reproductive performance than cows milked TAD. Moreover, cows milked OAD had a higher mean 3-week submission (by 7.7%), conception rate (by 7.9%), 6-week-in-calf rate (by

10.4%), herd 6-week calving rate (by 4.3%) and low percent of not in-calf rate (by 4.8%) compared to cows milked TAD for the whole season.

Negative energy balance (NEB) during the first 3-4 weeks of lactation, is highly correlated with the interval to first ovulation, severe NEB causes a longer interval to first ovulation and delays the conception during the breeding period (Butler, 2000). Many studies demonstrated that OAD milking could be used to better manage the period of NEB of cows during early lactation (Bernard et al., 2002; McNamara et al., 2008; Kay et al., 2013; Stelwagen et al., 2013). Increasing milking frequency in early lactation causes a greater NEB due to increased mobilisation of body reserves in support of milk production (Phyn et al., 2010).

A study by Rhodes et al. (1998) investigated the rate of resumption of oestrus cycles, and fertility to the first insemination of anoestrous cows before the planned start of mating in OAD and TAD milking cows. According to their findings, 65.8% and 76.6% of cows were detected in oestrus in TAD and OAD, respectively. OAD milking increased the rate of spontaneous resumption of oestrous cycles in anoestrous cows compared with TAD milking.

Table 2.4. Mean values for fertility traits[1] from dairy cows milked once daily (OAD) or twice daily (TAD) during the entire lactation in New Zealand

Study	Years	Breed[2]	Milking frequency	Fertility traits[1]							
				SMCO (days)	SR21 (%)	PR21 (%)	PR42 (%)	PR (%)	NIC (%)	CR21 (%)	CR42 (%)
Clark et al. (2006)	2000-2004	F	TAD	28.2	79.3	37.4	67.3	-	-	-	-
			OAD	26.1	89.7	41.8	69.8	-	-	-	-
		J	TAD	28.4	90.1	39.3	67.6	-	-	-	-
			OAD	23.8	94.4	50.4	75.1	-	-	-	-
Edwards (2018)	2007-2016	F, J, F×J	TAD	-	-	-	-	-	-	59.0	82.0
			OAD	-	-	-	-	-	-	64.0	87.0
Hemming et al. (2018)	2014-2016	F, J, F×J	TAD	-	76.9	-	64.4	52.2	17.6	-	83.9
			OAD	-	84.6	-	74.8	60.1	12.8	-	88.2

[1] SMCO = start of mating to conception (d), SR21 = cows inseminated in the first 21 days from the start of mating, PR21 = cows conceived in the first 21 days from the start of mating, PR42 = cows conceived in the first 42 days from the start of mating, PR = overall pregnancy rate at end of the breeding season, CR21 = cows that calved in the first 21 days from the planned start of the calving, CR42 = cows that calved in the first 42 days from the planned start of the calving.

[2] F = Holstein-Friesian, J = Jersey, F×J = crossbred Holstein-Friesian×Jersey.

2.7 Fertility performances of part of the lactation OAD milking cows in New Zealand

The effects of short-, medium- and long-term OAD on milk production, milk composition, processability characteristics, energy balance and cow health have been extensively researched in New Zealand grazing systems (Davis et al., 1999; Stelwagen et al., 2013; Lembeye et al., 2016b). Short-term OAD milking has gained considerable interest as a management strategy. Some dairy herds were switched to OAD milking, either in early or late lactation due to the diminished milk yield in early lactation (spring) and cows in metabolic stress during the period of feed shortages (Kay et al., 2013). In late lactation, farmers use OAD milking to manage the cow's condition as well as the preference of the farmer's lifestyle (Davis et al., 1999). According to the statistics from over 7000 Fonterra suppliers in New Zealand, 40% of farmers used OAD milking at some part of the lactation during the 2021-2022 season and many of these part-time OAD farms are post-Christmas OAD (Dairy NZ, 2023). However, fertility outcomes associated with the part-of the lactation OAD milking cows, especially cows milked in post-Christmas OAD have not been investigated in New Zealand.

2.8 Breed effects for milk production and fertility

Breed differences in milk production were identified in New Zealand dairy cows (Ahlborn-Breier and Hohenboken, 1991; Lembeye et al., 2015; Sneddon et al., 2015b). Lembeye et al. (2016d) documented that milksolids yields (kg per cow) were slightly greater in F and F×J compared to J cows. However, J cows were more efficient per 100 kg of live weight than F or F×J cows. Studies by Grosshans et al.

(1997) and Xu and Burton (2003) identified breed differences in reproductive performances. Grosshans et al. (1997) reported first and second parity J cows had superior fertility than F cows with shorter intervals for SMFS, SMCO, CFS, DO, CI and higher PR21, PR42. Likewise, Xu and Burton (2003) reported that J cows were mated earlier in the breeding season and had higher SR21 than F cows (J = 87% vs F = 83%) however, they reported that PRFS was lower in J cows than F cows (53% vs 56%) and no differences were found between F and J cows for pregnancy rates (PR42; 70% and final in-calf rate 90%).

A study by Lembeye et al. (2016c) reported that breed effects (F-J) on production differed across milking frequencies in New Zealand. They found that breed effects (F-J) increased with production levels in both OAD and TAD, but the increase was greater in TAD milking systems. Furthermore, authors suggested that F cows are better suited to high-production level environments milked TAD, in contrast with J cows which are better adapted to low–medium production level environments and to being milked OAD. Another study by Lembeye et al. (2016d) documented J cows milked OAD for entire lactation were more efficient per kg of live weight than F and F×J cows milked OAD. However, the authors suggested that F×J cows can be more suitable for OAD milking due to greater milksolids production, intermediate feed conversion efficiency and biological efficiency. Breed effects (F-J) for fertility traits are unknown in OAD milking cow population.

2.9 Heterosis effects for production and fertility traits

In 1955, the predominant breed in New Zealand dairy herds was J (75%) and imported genetic material from North American Holsteins was used in New Zealand herds from the late 1960s (Harris and Kolver, 2001). Since 1960, J cows were crossbred, aiming to upgrade J to F and after 1985 crossbreeding has been adopted as a mating strategy (Montgomerie 2005). The breed mix has continued to change in past decades and at present, the crossbreed of F×J is the dominant breed type in New Zealand and it accounts more than half (59.2%) of the national herd (LIC and DairyNZ, 2022).

Favourable heterosis for production, reproduction and survival traits leads to an increased overall farm profitability (Lopez-Villalobos et al., 2000). Favourable heterosis effects for production traits have been reported with cows milked TAD and OAD in New Zealand (Ahlborn-Breier and Hohenboken, 1991; Harris, 2005; Lembeye et al., 2015). The reported heterosis effects in the first cross of F and J cows milked TAD for yields of milk, fat and protein ranged from 4.92-6.1%, 7.39-7.7% and 5.5-6.21% in the previously cited literature, respectively. Though the heterosis effects for milk production traits were well understood in New Zealand dairy cattle milked TAD, only limited research has been done to investigate the heterosis effect for production traits under OAD milking. Lembeye et al. (2015) reported heterosis effects of first-cross F×J cows milked OAD as 5.49% for milk yield (MY), 6.84% for fat yield (FY) and 6.49% for protein yield (PY).

Earlier, Ahlborn-Breier and Hohenboken (1991) estimated individual and maternal breed and heterosis effects for yields of milk, fat, and fat percentage (FP) for New

Zealand dairy cows milked TAD. Estimated maternal heterosis effects for MY, FY and FP were -2 kg, -1.1 kg and -0.05%, respectively.

Several studies have indicated favourable heterosis effects exist in fertility traits of dairy cows in New Zealand and other dairy cattle populations (Table 2.5). The studies by Harris et al. (2001) and Harris and Pryce (2004) estimated heterosis effects for fertility traits in New Zealand dairy cattle using a regression model. Harris et al. (2001) considered four fertility traits, SMFS, a calf born from AB, 2-24 days non-return and 18-24 days non-return. They found first-cross F×J cows had reduced SMFS (1.1 days) and higher calving rate to successful AB (3.4%) than the mean performances of their parental F and J breeds. Harris and Pryce (2004) also documented favorable heterosis effects on the fertility of first-cross F×J, with the average probability of a cow to be mated in the first 21 days of mating and the average probability of a cow to have a calf born to AB being above the average means of their parent breeds.

Studies by Harris and Kolver (2001) and Harris et al. (2006) reported that first-cross F×J cows had 1.5 days shorter SMFS, 10.1% greater pregnancy rate, 4.3% greater SR21, and 3.4% greater CR42 compared with the mean performances of the parental F and J breeds in New Zealand. The average heterosis effect for cow's fertility was discussed by Montgomerie (2005) for first-cross F and J cows. A review by Harris (2005) summarised the findings of Montgomerie (2005) and reported the heterosis estimate of the cow's fertility as 3.4%.

The importance of the heterosis effect on the fertility performances of dairy cows milked TAD is well established, but so far, no studies have quantified heterosis effects on reproductive traits of cows milked OAD. Furthermore, maternal heterosis effects for fertility traits have not been estimated in New Zealand dairy cattle.

Table 2.5. Published estimated heterosis[1] effects for fertility traits[2] in previous studies

Reference	Country[3]	Breed[4]	Days							SC	Rates (%)				
			AFS	AFC	SMFS	CFS	FSCO	DO	CI		SR21	PRFS	PR42	CR42	CAI
Harris et al. (2001)	NZ	F×J	-	-	-1.1	-	-	-	-	-	-	-	-	-	3.40
Harris and Kolver (2001)	NZ	NZF×J	-	-	-1.2	-	-	-	-	-	-	-	-	-	6.80
		OSH×J	-	-	-1.5	-	-	-	-	-	-	-	-	-	10.1
Harris et al. (2006)	NZ	F×J	-	-	-	-	-	-	-	-	4.30	-	-	3.40	-
Lopez-Villalobos (1998)[5]	-	-	-0.02	0.01	-	0.04	-	-5.8	-0.8	0.09	-	-	-	-	-
Penasa et al. (2010)	Ireland	OSHF×FR	-	-	-	-	-	-	-1.3	-	-	-	-	-	-
		OSHF×J	-	-	-	-	-	-	-2.0	-	-	-	-	-	-
		OSHF×M	-	-	-	-	-	-	-10.2	-	-	-	-	-	-
Coffey et al. (2016)	Ireland	H×FR	-	-1.30	-	2.40	-	-	-5.5	-	-4.55	-	-	3.31	-
		H×J	-	-11.7	-	-0.40	-	-	-7.6	-	3.17	-	-	-3.64	-
		FR×J	-	-2.90	-	-1.80	-	-	-9.3	-	0.88	-	-	-9.46	-
Kelleher et al. (2016)	Ireland	H×FR	-1.60	-5.83	-	-0.90	-	-	-6.68	-0.05	0.02	0.03	0.07	0.02	-
		H×J	-2.38	-6.90	-	-1.40	-	-	-9.42	-0.05	0.04	0.05	0.06	0.01	-
		H×M	-10.0	-28.3	-	-1.08	-	-	-9.56	-0.05	0.05	0.05	0.06	0.02	-
		FR×J	-0.56	-3.58	-	0.11	-	-	-9.18	-0.08	0.01	0.05	0.07	0.02	-
		FR×M	-16.2	-28.3	-	-0.11	-	-	-7.92	-0.02	-0.02	0.04	0.06	0.04	-
		J×M	-16.8	-22.9	-	2.82	-	-	-11.8	-0.02	-0.04	0.09	0.001	0.08	-
Wall et al. (2005)	UK	H×FR	-	-	-	-1.14	-	-	-1.61	-0.02	-	-	-	-	-
Dechow et al. (2007)	US	H×BS	2.06	-	-	-	-	7.99	-	-	-	-	-	-	-
Sorensen et al. (2008)[5]	Denmark	-	-	-	-	-1.40	-11.1	-	-	0.02	-	-0.40	-	-	-
Dezetter et al. (2015)	France	H×M	-	-	-	-3.0	-	-	-	-	-	-	-	-	3.0
		H×N	-	-	-	-6.0	-	-	-	-	-	-	-	-	2.0

[1] Heterosis is expressed as the difference between the average of reciprocal first crosses and the average of straightbred cows.

[2] AFS = Age at first service, AFC = age at first calving, SMFS = start of mating to first service, CFS = calving to first service, FSCO = first service to conception, DO = calving to conception/days open, CI = consecutive calving interval, SC = number of services per conception, SR21 = cow inseminated in the first 3 wk from the start of mating, PRFS = cow pregnant to first service, PR42 = cow conceived in the first 6 wk from the start of mating, CR42 = cow calved in the first 6 wk from the planned start of the calving, CAI = cow in-calf to AI.

[3] NZ = New Zealand, UK = United Kingdom, US = United States.

[4] F×J = crossbred Holstein-Friesian×Jersey, NZF×J = New Zealand Holstein-Friesian×Jersey, OSH×J = North American Holstein×Jersey, OSHF×FR = North American Holstein-Friesian×Friesian, OSHF×J = North American Holstein-Friesian×Jersey, OSHF×M = North American Holstein-Friesian×Montbeliarde, H×FR = Holstein× Friesian, H×J = Holstein×Jersey, FR×J = Friesian×Jersey, H×M = Holstein×Montbeliarde, FR×M = Friesian×Montbeliarde, J×M = Jersey×Montbeliarde, H×BS = Holstein×Brown Swiss, H×N = Holstein×Normande.

[5] Represents results derived from the meta-analysis and detailed studies presented with reference.

2.10 Recombination loss effects for milk production and fertility traits

Recombination loss is defined as the breakup of epistatic effects during meiosis to form nonparental interlocus combinations of alleles in gametes of crossbred parents (Dickerson, 1973). To precisely estimate recombination loss effects, a large dataset with more second- and greater-generation crosses is required (Dechow et al., 2007). Lopez-Villalobos (1998) extensively summarized the crossbreeding research with recombination loss effects on production traits in different dairy breeds in temperate climates. In general, the review demonstrated that the estimates of recombination losses for yield traits were large and negative whereas those for fat and protein percentages were low and non-significant for most of the crosses.

There is conflicting evidence for the direction of recombination effects in the literature (Table 2.6). Coffey et al. (2016) and Kelleher et al. (2016) reported recombination loss effects for production and fertility traits were favourable with large standard errors in Irish dairy cattle. In contrast, Dechow et al. (2007) and Dezetter et al. (2015) reported unfavourable recombination effects for production and fertility traits. Reported recombination effects for fertility traits were small but generally not different from zero in the literature (Table 2.6). Due to the large proportion of crossbred F×J cows in the national herd, it is important to investigate the recombination effects for milk production and fertility. However, there is a paucity of information available in the literature about the recombination effects of production and fertility traits of crossbred F×J cows in New Zealand. Moreover, recombination effects for milk production and fertility traits have not been estimated in the OAD milking cow population.

Table 2.6. Published recombination loss effects (standard errors) for milk production[1] and fertility[2] traits in previous studies

			Milk production[1]					Fertility[2]						
Reference	Country[3]	Breed[4]	MY (kg)	FY (kg)	PY (kg)	FP (%)	PP (%)	CFS (days)	CI (days)	SR21 (%)	PRFS (%)	PR42 (%)	PR (%)	CR42 (%)
Coffey et al. (2016)	Ireland	H×FR	820* (123)	46.3* (5.0)	28.6* (4.1)	0.02 (0.06)	0.05* (0.03)	1.0 (2.0)	1.8 (5.9)	1.3 (-4.1 to 6.1)	-	-	-	3.9 (-8.5 to 13.7)
		H×J	22.6 (147)	4.9 (6.0)	2.9 (5.0)	0.01 (0.07)	0.05 (0.03)	-3.2 (2.3)	-16.7* (7.1)	4.8 (-1.6 to 10)	-	-	-	-15.3 (34.3 to 3.4)
		FR×J	625 (333)	22.5 (13.6)	20.3 (11.2)	0.05* (0.02)	-0.04 (0.07)	12.0* (4.8)	-2.6 (15.9)	-16.2* (-35.9 to 0.3)	-	-	-	-16.7 (-49.4 to 22)
Kelleher et al. (2016)	Ireland	H×FR	-	-	-	-	-	-0.5 (0.8)	-3.2 (2.6)	0.03 (0.02)	0.06 (0.03)	0.03 (0.02)	-	−0.01 (0.01)
		H×J	-	-	-	-	-	-1.2 (1.1)	-9.2* (3.8)	0.01 (0.03)	0.08 (0.06)	0.03 (0.03)	-	−0.01 (0.02)
		H×M	-	-	-	-	-	0.5 (1.6)	-3.1 (5.2)	0.08* (0.04)	0.08 (0.07)	0.03 (0.05)	-	0.07* (0.03)
		FR×J	-	-	-	-	-	0.8 (2.4)	-15.4 (8.6)	−0.07 (0.06)	−0.06 (0.13)	0.003 (0.07)	-	0.01 (0.05)
		FR×M	-	-	-	-	-	-7.8 (4.5)	8.3 (14.4)	0.09 (0.12)	−0.01 (0.2)	0.10 (0.14)	-	−0.01 (0.10)
		J×M	-	-	-	-	-	1.2 (9.3)	3.3 (36.5)	0.04 (0.24)	0.54 (0.4)	0.21 (0.3)	-	−0.13 (0.21)
Wall et al. (2005)	UK	H×FR	-1.45* (0.09)	-	-	-	-	-0.04 (0.6)	-1.3 (1.2)	-	-	-	-	-
Dechow et al. (2007)	US	H×BS	-3.49	-3.76*	-3.31*	-	-	-	-	-	-	-	-	-
Dezetter et al. (2015)	France	H×M	-183 (129)	11 (5.2)	-7.0 (4.0)	-0.02 (0.04)	-0.03 (0.02)	0.3 (3.1)	-	-	-	-	-5.0 (2.8)	-
		H×N	-128 (113)	-6.0 (4.6)	-3.0 (3.6)	-0.08 (0.03)	-0.06 (0.02)	-4.0 (4.6)	-	-	-	-	-4.0 (2.7)	-

[1] MY = Milk yield, FY = fat yield, PY = protein yield, FP = fat percentage, PP = protein percentage.

[2] CFS = calving to first service, CI = consecutive calving interval, SR21 = cow inseminated in the first 3 wk from the start of mating, PRFS = cow conceived to first service, PR42 = cow conceived in the first 6 wk from the start of mating, PR= overall pregnancy rate, CR42 = cow calved in the first 6 wk from the planned start of the calving.

[3] UK = United Kingdom, US = United States.

[4] H×FR = Holstein× Friesian, H×J = Holstein×Jersey, FR×J = Friesian×Jersey, H×M = Holstein×Montbeliarde, FR×M = Friesian×Montbeliarde, J×M = Jersey×Montbeliarde, H×BS = Holstein×Brown Swiss, H×N = Holstein×Normande.

* Represents significant recombination loss effects ($p<0.05$).

2.11 Genetic parameters for production and reproductive measures

Accurate estimates of genetic parameters including heritability, genetic and phenotypic correlations for milk production traits are essential to predict the response to selection for traits or a combination of traits (López-Villalobos, 2012). The heritability (h^2) of a trait indicates the proportion of the phenotypic variation attributable to genetic-additive differences among animals (Berry et al., 2014). In notations heritability (h^2) is calculated as $h^2 = \sigma_a^2/\sigma_p^2$ where σ_a^2 and σ_p^2 are additive genetic variance and phenotypic variance, respectively (Falconer and Mackay, 1996). When repeated measures are presented for the same trait, another source of variation is considered as permanent-environmental variance, which is partly non-additive genetic and partly environmental variance (Falconer and Mackay, 1996). Therefore, h^2 and repeatability (t) estimates can be expressed as $h^2 = \sigma_a^2/(\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ and $t = (\sigma_a^2 + \sigma_c^2)/(\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$, respectively, where σ_a^2 is additive genetic variance, σ_c^2 is the cow permanent environmental variance and σ_e^2 is residual variance.

2.11.1 Heritability and repeatability estimates for milk production and fertility traits

The estimation of heritability for milk production traits has been extensively studied in New Zealand in TAD milking dairy cows (Ahlborn and Dempfle, 1992; Harris and Pryce, 2004; Pryce and Harris, 2006; Bryant et al., 2007; Sneddon et al., 2015a; Lembeye et al., 2016a). In general, the previously cited literature reported the heritability estimates for MY with a range of (0.22-0.36), FY (0.19-0.29), PY (0.16-0.29), LY (0.18), FP (0.35-0.66) and PP (0.32-0.67) in TAD milking system. A study by Lembeye et al. (2016a) estimated heritability for yields and milk composition traits considering both OAD and TAD milking cows in New Zealand. They reported heritabilities for yields and composition traits were consistent between milking frequencies (MY: 0.36 vs 0.33, FY: 0.26 vs 0.21, PY: 0.25 vs 0.22, FP: 0.66 vs 0.62 and PP: both were 0.67).

In New Zealand the two binary fertility traits SR21 and CR42 are the most researched, since those are the key traits used for national genetic evaluation in New Zealand dairy cows (Harris et al., 2006). A continuous trait, calving season day (CSD), which is the number of days that the cow calved from the planned start of calving has also been introduced lately for national genetic evaluations (Bowley et al., 2015; Amer et al., 2016; Craig et al., 2018). A summary of heritability estimates reported with fertility traits of New Zealand dairy cows is presented in Table 2.7. In general, all studies estimated the heritability for fertility traits at less than 10% and the two fertility traits of SR21 and CR42 ranged from 0.02 to 0.07 and 0.004 to 0.029, respectively.

Studies based on Irish, Australian, United Kingdom and United States dairy cattle populations highlighted that dairy cattle reproductive traits tend to be lowly heritable (Dematawewa and Berger, 1998; Pryce et al., 1998; Berry et al., 2003; Haile-Mariam et al., 2003). Berry et al. (2014) indicated that heritability estimates derived from interval traits fertility measures had greater heritability than either estimate derived from binary or count traits. Grosshans et al. (1997) documented that heritability estimators derived through the start of the breeding season (SMFS, SMCO, PR21, PR42) had relatively higher heritability than the estimates derived from calving dates (CFS, CI). A low heritability indicates that a relatively slow improvement in fertility would result from selection (Short et al., 1990).

Table 2.7. Heritability estimates (standard errors) for fertility traits[1] in New Zealand dairy cattle

References	Years	Breed[2]	Cows	Traits	Heritability (standard error)			
					1st lactation	2nd lactation	3rd lactation	All lactations
Grosshans et al. (1997)	1986-1992	F, J	66, 294	SMFS	0.06(0.006)	0.06(0.008)	-	-
				SMCO	0.04(0.005)	0.04(0.006)	-	-
				CFS	0.03(0.005)	0.01(0.004)	-	-
				FSCO	0.01(0.003)	0.01(0.003)	-	-
				DO	0.02(0.004)	0.01(0.003)	-	-
				CI	0.02(0.004)	0.01(0.003)	-	-
				AC	0.13(0.011)	0.12(0.004)	-	-
				SC	0.01(0.005)	0.01(0.003)	-	-
				PR21	0.04(0.005)	0.03(0.005)	-	-
				PR42	0.03(0.004)	0.03(0.005)	-	-
Harris and Pryce (2004)	2000-2003	NZF, OSHF, J, NZF×J, OSHF×J, NZF×OSHF	99,870	SR21	0.049	-	-	-
				CAI	0.020	-	-	-
Harris et al. (2005)	1990-2004	F (52%), J (22%), A (1%), F×J (25%)	221,224	SR21	0.05	0.05	0.036	-
				CR42	0.022	0.026	0.015	-
Pryce and Harris (2006)	2000-2001 2003-2004	F, J, F×J	169,661	SR21	0.068	-	-	-
				CR42	0.029	-	-	-
Pryce et al. (2007)	1989-2003	NZF, OSHF, J, NZF×J, OSHF×J, NZF×OSHF	244,453	SR21	0.070(0.005)	-	-	-
				CR42	0.027(0.003)	-	-	-
Bowley et al. (2015)	2011-2012	F, J, F×J	259,651	PPAI	-	-	-	0.0814 (0.0104)
				SR21	-	-	-	0.0335 (0.0035)
				MSD	-	-	-	0.0239 (0.0029)
				CR42	-	-	-	0.0087 (0.0015)
				CSD	-	-	-	0.0206 (0.0027)

Table 2.7. Heritability estimates (standard errors) for fertility traits[1] in New Zealand dairy cattle (continued)

References	Years	Breed[2]	Cows	Traits	Heritability (standard error)			
					1st lactation	2nd lactation	3rd lactation	
Amer et al. (2016)	1989-2013	F, J, F×J	53,614	SR21	0.031	0.058	0.052	-
				CSD	0.016	0.013	0.019	-
Craig et al. (2018)*	1997-2009	F, J, F×J	3,017,535	SR21	-	0.023(0.007)	0.020(0.007)	-
				CSD	0.012(0.005)	0.016(0.006)	0.018(0.007)	-
				CR42	0.008(0.004)	0.004(0.004)	0.008(0.005)	-

[1] SR21 = cow inseminated in the first 3 wk from the start of mating (yes/no), CAI = cow in-calf to AI (probability), CR42 = cow calved in the first 6 wk from the planned start of the calving (days), PPAI = postpartum anestrous interval (days), MSD = mating season day (days), CSD = calving season day (days).

[2] F = Holstein-Friesian, J = Jersey, NZF = New Zealand Holstein Friesian, OSHF = North American Holstein Friesian, NZF×J = New Zealand Holstein Friesian×Jersey, OSHF×J = North American Holstein Friesian×Jersey, NZF×OSHF = New Zealand Holstein Friesian×North American Holstein Friesian, A = Ayrshire, F×J = crossbred of Holstein-Friesian×Jersey. *Heritability represents only for high fertility dairy herds.

Repeatability estimates for milk production traits have been documented in New Zealand dairy cattle (Sneddon et al., 2015a; Lembeye et al., 2016a; Lopez-Villalobos et al., 2020). However, there is a paucity of information on repeatability estimates of fertility traits in New Zealand dairy cows. In general, repeatability estimates for MY, FY, PY and LY varied between 0.39 and 0.52 and repeatabilities for FP, PP and LP were comparatively higher than yield traits (ranging from 0.46 to 0.80) in the previously cited literature. Lembeye et al. (2016a) examined the repeatability estimates for yields and composition traits with OAD milking cows in New Zealand. According to their findings estimates of the repeatabilities were similar between milking frequencies, but slightly greater in TAD milking population than in OAD milking cow population.

2.11.2 Genetic and phenotypic correlations between production and fertility traits

Estimates of genetic and phenotypic correlations between milk production and fertility traits have been reported in seasonal dairy-production systems (Grosshans et al., 1997; Pryce et al., 1998; Berry et al., 2013; Morton et al., 2018). However, most reported genetic parameters for production and fertility traits are associated with data from cows milked TAD. The main conclusion from the cited studies is that genetic correlations between milk production and fertility traits are antagonistic. Genetic correlation estimates reported between milk production and fertility traits in New Zealand dairy cows have been summarised in Table 2.8.

Table 2.8. Published genetic correlations (standard error) between fertility[1] and production[2] traits in New Zealand studies

Source	Traits	First lactation					Second lactation		
		MY (kg)	FY (kg)	PY (kg)	FP (%)	PP (%)	MY	FY	PY
Grosshans et al. (1997)	SMFS	0.25	0.13	0.17	-	-	0.19	0.08	0.07
	SMCO	0.28	0.16	0.16	-	-	0.34	0.18	0.22
	CFS	0.25	-0.04	-0.01	-	-	-0.25	-0.34	-0.33
	FSCO	0.16	0.11	0.06	-	-	0.33	0.26	0.27
	DO	0.25	0.04	0.04	-	-	0.17	0.04	0.05
	CI	0.22	0.03	0.07	-	-	-0.03	-0.14	-0.20
	AC	0.21	0.45	0.70	-	-	0.24	0.32	0.31
	SC	0.17	0.29	0.19	-	-	0.26	0.28	0.27
	PR21	-0.26	-0.22	-0.25	-	-	-0.29	-0.15	-0.12
	PR42	-0.19	-0.18	-0.17	-	-	-0.41	-0.24	-0.33
Harris and Pryce (2004)	SR21	-0.25(0.05)	-	-	0.19(0.05)	0.18(0.05)	-	-	-
	CAI	-0.21(0.09)	-	-	0.32(0.09)	0.20(0.09)	-	-	-
Harris et al. (2005)	SR21	-0.17	-	-	-	-	-0.23	-	-
	CR42	-0.16	-	-	-	-	-0.17	-	-
Pryce and Harris (2006)	SR21	-0.23(0.04)	-0.10(0.04)	-0.13(0.04)	-	-	-	-	-
	CR42	-0.15(0.05)	-0.06(0.05)	-0.05(0.05)	-	-	-	-	-
Pryce et al. (2007)*	SR21	-0.18(0.04)	-	-	-	-	-	-	-
	CR42	-0.18(0.05)	-	-	-	-	-	-	-

[1] SMFS = Start of mating to first service (days), SMCO = start of mating to conception (days), CFS = calving to first service (days), FSCO = first service to conception (days), DO = calving to conception/days open (days), CI = consecutive calving interval (days), AC = age at calving (days), SC = number of services for conception, PR21 = cow conceived in the first 3 wk from the start of mating (yes/no), PR42 = cow conceived in the first 6 wk from the start of mating (yes/no), SR21 = cow inseminated in the first 3 wk from the start of mating (yes/no), CAI = cow in-calf to AI (probability), CR42 = cow calved in the first 6 wk from the planned start of the calving (yes/no).

[2] MY = Milk yield, FY = fat yield, PY = protein yield, FP = fat percentage, PP = protein percentage.

Generally, all the fertility traits were unfavourably correlated with milk production and genetic correlations between MY and SR21 and CR42 ranged from -0.17 to -0.25 and -0.15 to -0.18, respectively. Pryce et al. (2014) suggested that in an environment where there is a strong genetic correlation between MY and reproductive performance, more emphasis is required to be placed on selection for fertility to prevent deterioration in this trait.

Estimation of genetic and phenotypic correlations amongst the fertility traits is also important in seasonal calving dairy herds since all the management operations are synchronized with calving and mating in the subsequent year. For instance, two fertility traits, the interval from the SMFS and CR42 are negatively correlated. A longer interval from SMFS is expected to reduce CR42. Therefore, studying genetic and phenotypic correlations between fertility traits allows an understanding of these relationships and how they can be used in genetic evaluations. Grosshans et al. (1997) reported that the genetic and phenotypic correlations among fertility traits were moderate to strong in first and second-lactation dairy cows in New Zealand. They found all interval fertility traits were positively correlated with each other and all interval fertility traits were negatively correlated with PR21 and PR42. Though these relationships are well documented with TAD milking cow population there are not published estimates in the OAD milking population.

Energy balance in early lactation is closely linked with the reproductive performances of dairy cows and breeds selected for high production are more likely to be in extreme negative energy balance in early lactation (Pryce et al., 2004). There is evidence that OAD milking can improve the energy balance of dairy cows in early lactation compared with TAD milking (Phyn et al., 2011; Kay et al., 2013). Previous studies have investigated the phenotypic reproductive performance of OAD milking cows in New Zealand dairy herds (Edwards, 2018; Hemming et al., 2018). However, no study has been conducted to estimate the heritability, repeatability for fertility traits and genetic and phenotypic correlations between milk production and fertility traits in OAD milking cow population in New Zealand.

2.12 Genomics for milk composition and fertility traits

Genomic selection is a form of marker assisted selection where the breeding values of individuals are calculated based on genomic information (Meuwissen et al., 2001). Genomic selection is used in dairy cattle breeding programs to improve the rate of genetic gain by increasing the accuracy of genomic breeding values in young animals (Goddard and Hayes, 2007; Hayes et al., 2009; Meuwissen et al., 2013) and therefore reducing the generation intervals (Schaeffer, 2006). Since 2008, genomically enhanced breeding values have been used for genomic selection in New Zealand dairy cattle (Harris et al., 2008; Winkelman et al., 2015).

Numerous studies have identified candidate genes associated with milk composition traits in New Zealand and other dairy cattle populations (Ashwell et al., 2004; Meredith et al., 2012; Lu et al., 2020; Wang et al., 2020; Tiplady et al., 2021). Also, GWAS have been conducted to discover genomic regions associated with diverse fertility traits in many dairy cattle populations (Cole et al., 2011; Berry et al., 2012; Minozzi et al., 2013; Höglund et al., 2015; Nayeri et al., 2016; Parker Gaddis et al., 2016). In contrast, some studies did not identify significant associations with dairy cattle fertility (Pimentel et al., 2011; Nayeri et al., 2017).

Most reported markers and genes, with their functions for milk composition and fertility traits in literature are presented in Table 2.9 and Table 2.10, respectively. Though many associations were detected with milk composition traits, generally fewer associations have been identified with fertility traits. Due to the polygenic nature and low heritability of fertility traits, many GWAS of dairy fertility tend to be underpowered (Ma et al., 2019) and fertility traits are highly affected by environmental factors, which are difficult to quantify (Wolf et al., 2021). Furthermore, many fertility measures are not related to biological processes that determine dairy cow's fertility, but they are correlated measures that are also affected by management decisions (Minozzi et al., 2013).

The candidate genes and biological functions associated with fertility traits with seasonally calved dairy cows are scarce in the literature. Importantly, to date no published GWAS have reported candidate genes associated with the key fertility traits of SR21, PR42, NIC and CR42 in a seasonal calving system.

Table 2.9. Genome-wide association studies reporting genes and markers associated with percentage of fat (FP), protein (PP), lactose (LP) traits in dairy cows

Trait	Gene abbreviation	Candidate gene	Chr[1]	Function	Reference
FP, PP	ADCK5	aarF domain containing kinase 5	14	Unknown	Ibeagha-Awemu et al. (2016), Wang et al. (2020)
	AGO2	argonaute RISC catalytic component 2	14	miRNA processing, miRNA metabolic process,	Bakhshalizadeh et al. (2021)
	CPSF1	cleavage and polyadenylation specific factor 1	14	mRNA polyadenylation	Ibeagha-Awemu et al. (2016), Ariyarathne et al. (2021), Tiplady et al. (2021)
	DGAT1	diacylglycerol O-acyltransferase 1	14	Diacylglycerol metabolic process, fatty acid homeostasis, lipid storage, long-chain fatty-acyl-CoA metabolic process, triglyceride biosynthetic process	Nayeri et al. (2016), Ariyarathne et al. (2021), Tiplady et al. (2021)
	FOXH1	forkhead box H1	14	Lipid metabolism, negative regulation of DNA-binding transcription factor activity.	Nayeri et al. (2016), Cruz et al. (2019), Wang et al. (2020)
	IQANK1	IQ motif and ankyrin repeat containing 1	14	Regulation of barbed-end actin filament capping	Xiang et al. (2022)
	MAPK15	mitogen-activated protein kinase 15	14	Protein phosphorylation	Wang et al. (2020), Bakhshalizadeh et al. (2021)
	MROH1	maestro heat like repeat family member 1	14	Unknown	Sanchez et al. (2017), Wang et al. (2020), Tiplady et al. (2021)
	RHPN1	rhophilin Rho GTPase binding protein 1	14	Signal transduction, negative regulation of stress fiber assembly	Kirkpatrick et al. (2022)
	SLC52A2	solute carrier family 52 member 2	14	Riboflavin transport	Wang et al. (2020), Ariyarathne et al. (2021), Tiplady et al. (2021)

Table 2.9. Genome-wide association studies reporting genes and markers associated with percentage of fat (FP), protein (PP), lactose (LP) traits in dairy cows (continued)

Trait	Gene abbreviation	Candidate gene	Chr[1]	Function	Reference
FP, PP	TRAPPC9	trafficking protein particle complex subunit 9	14	Positive regulation of NF-kappaB transcription factor activity	Bakhshalizadeh et al. (2021)
	ZC3H3	zinc finger CCCH-type containing 3	14	mRNA polyadenylation	Bakhshalizadeh et al. (2021)
FP	MGST1	Microsomal glutathione S-transferase 1	5	Regulation of fat synthesis, cellular response to lipid hydroperoxide	Ariyarathne et al. (2021), Tiplady et al. (2021)
PP	CSN3	casein kappa	6	Response to dehydroepiandrosterone, response to 11-deoxycorticosterone, response to progesterone, response to alcohol, response to estradiol, response to oxygen-containing compound, response to organic cyclic compound, response to lipid, gland development, lactation	Sanchez et al. (2017), Zhou et al. (2019)
PP, LP	KCNH4	potassium voltage-gated channel subfamily H member 4	19	Ion transport, potassium ion transport, regulation of ion transmembrane transport	Lopdell et al. (2017), Tiplady et al. (2021)
	PICALM	phosphatidylinositol binding clathrin assembly protein	29	Vesicle transport and assembly as a clathrin recruitment protein	Lopdell et al. (2017), Sanchez et al. (2017), Tiplady et al. (2022)
LP	STAT5B	signal transducer and activator of transcription 5B	19	Controlling milk protein gene expression, including lactalbumin for lactose synthesis, affect mammary gland development, prolactin signaling, and mammary cell involution	Lopdell et al. (2017), Lu et al. (2020)

[2] Chr = chromosome

Table 2.10. Genome-wide association studies reporting genes and markers associated with fertility traits[1] in dairy cows

Reference	Country	Trait[1]	Gene abbreviation	Candidate gene	Chr[2]	Function
Berry et al. (2012)	Ireland, United Kingdom, Netherlands, Sweden	CFS	-	-	6, 20	-
		CI	-	-	19	-
		SC	-	-	4	-
		PRFS	-	-	13	-
		CLA	ARRDC4	Bos taurus arrestin domain containing 4	2	Neurological processes
			ID3	Bos taurus inhibitor of DNA binding 3, dominant negative helix-loop-helix protein	21	Growth of vascular cells through signalling that is induced by oestrogen
			FUCA1	Bos taurus fucosidase, alpha-L-1, tissue	21	Carbohydrate and fucose metabolic process, glycoside catabolic process
			E2F	Transcription factor 2	21	Control of cell cycle and action of tumour suppressor proteins
Minozzi et al. (2013)	Italy	CFS	IGF1	Insulin-like growth factor 1 gene	5	-
			TGFB2	transcription growth factor b2	16	Follicular development by interacting with the reproductive hormones LH and FSH
		CI	AMHR2	Anti-müllerian hormone receptor type II gene	5	Anti-müllerian hormone is an early follicle growth inhibitor
Höglund et al. (2015)	Denmark	CFS	PLA2G2F	Bos taurus phospholipase A2, group IIF	2	-
			KCNIP4	Bos taurus Kv channel interacting protein 4	6	Protein binding (GO:0005515)
			ANKRD60	Ankyrin repeat domain 60	13	Protein binding (GO:0005515)
			GRAMD1B	GRAM domain containing 1B	15	Unknown

Table 2.10. Genome-wide association studies reporting genes and markers associated with fertility traits[1] in dairy cows (continued)

Reference	Country	Trait	Gene abbreviation	Candidate gene	Chr[2]	Function
Nayeri et al. (2016)	Canada	CFS, DO	FAM181A	Family with sequence similarity 181 member A	21	Methylation of this gene increases during mid-secretory phase of progesterone
			SLC24A4	Solute carrier family 24 member 4	21	A member of potassium-dependent sodium or calcium exchanger protein family
			NKX2-1	NK2 homeobox 1	21	A transcription factor that regulates the expression of thyroid-specific genes and involved in morphogenesis
Parker Gaddis et al. (2016)*	United States	CR	AFF1	AF4/FMR2 family, member 1	6	A transcription factor in regulatory gene networks underlying puberty
			CCL19	Chemokine (C-C motif) ligand 19	8	Controlling lymphocyte populations that are associated with embryo implantation
			TMEM207	Transmembrane protein 207	1	-
			TPRG1	Tumor protein p63 regulated 1	1	-
			ZNF641	Zinc finger protein 641	5	-
			TRPC7	Transient receptor potential cation channel, subfamily C, member 7	7	-
			KIF27	Kinesin family member 27	8	-
			SLC28A3	Solute carrier family 28 (concentrative nucleoside transporter), member 3	8	-
			LAMC3	Laminin, gamma 3	11	-
			CELF1	CUGBP, Elav-like family member 1	15	-
			SMYD2	SET and MYND domain containing 2	16	-
			LRRC4B	Leucine rich repeat containing 4B	18	-
			UQCRC1	Ubiquinol-cytochrome c reductase core protein I	22	-
			HEPHL1	Hephaestin-like 1	29	-

Table 2.10. Genome-wide association studies reporting genes and markers associated with fertility traits[1] in dairy cows (continued)

Reference	Country	Trait	Gene abbreviation	Candidate gene	Chr[2]	Function
Tenghe et al. (2016)	Sweden	CFS	-	-	3	-
			-	-	15	-
			-	-	23	-
Wolf et al. (2021)	Germany	CFS	KLHL1	Kelch like family member 1	12	-
			ARHGAP21	Rho GTPase activating protein 21	13	-
			LIN7C	Lin-7 homolog C, crumbs cell polarity complex component	15	-
			ENSBTAG00000052005	Unknown	15	-
			OR5BE5	Olfactory receptor family 5 subfamily BE member 5	15	-
			CHAT	Choline O-acetyltransferase	28	-

[1] CFS = calving to first service, CI = consecutive calving interval, SC = number of services for conception, PRFS = pregnant to first service, CLA = commencement of luteal activity, DO = calving to conception/days open, CR= conception rate. [2] Chr = chromosome.

* Markers associated with cow conception rate (CR) from high density panel in the cow population were presented.

2.13 Summary and Implications

Dairy cattle fertility is a key trait that has a major impact on the seasonal calving dairy system and profitability of dairy farming operations. Once daily milking is becoming a popular milking practice among New Zealand dairy farmers, however, little is known about the differences in fertility performance of full- or part- lactation OAD milking cows in New Zealand. Furthermore, genetic parameters, individual and maternal breed, heterosis and recombination loss effects for fertility traits of cows milked OAD are not known. The genetic correlations between milk production and fertility traits could be different between OAD and TAD milking cow populations due to the reduced extent of negative energy balance in early lactation for cows milked in OAD compared with cows milked in TAD. It is possible to estimate the genetic correlations of milk production and fertility traits in OAD and TAD milking cows in New Zealand because common sires are used in both milking environments.

2.14 References

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Chapter 3

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

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3.1 Abstract

The objective of this study was to evaluate the reproductive performance of New Zealand dairy cows with different milking regimens. A total of 2562 herds calving in 2017 met the criteria for inclusion in this study. The herds were classified into five different milking regimens; 260 herds with cows milked once daily (OAD) during the entire lactation, 1206 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 analysed using survival analysis. Time from SMFS, SMCO and FSCO was significantly shorter in cows milked OAD compared to cows milked TAD. Also, cows milked OAD had fewer services per conception (SC) 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 to 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

3.2 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 impact 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 (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 3.1).

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 of 2015-2016, about 9% of herd-tested herds milked once daily

(OAD) for the whole lactation in New Zealand (Edwards, 2018). Farmers cite a number of benefits from changing to OAD milking. These include ease of attracting labour, reduced labour costs, management of feed shortfalls, herd expansion, allowing time to build capital, utilising farmland with hilly terrain or long walking distances, herd health and management (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., 2012).

Research on the impact of strategic OAD milking on cow fertility has been limited. Only three 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 not in-calf (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 impact 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 3-6 yr-old cows (predominantly 2nd-5th parity). Understanding the effect of parity on reproductive performance can help farmers identify cows that need extra 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.

3.3 Material and methods

3.3.1 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 of 2017-2018 (1-June 2017 to 31-May 2018) were extracted from Livestock Improvement Corporation (LIC), New Zealand. Initial data sets contained data from 4173 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 3823 herds. Herds with fewer than four 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 3725 herds.

3.3.2 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 date (PSC), the second usually between 120 d from the first herd test and 180 d from the PSC. The planned start of calving 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 two 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 two 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, two herd tests were completed before the mating period finished and cows were milked TAD at the first test date (prior to 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 25th of December 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-May 2018, and at least one TAD herd test occurred after 25th December 2017. Herds that could not be classified as either OAD, TAD, OAD-M, OAD-P or OAD-E were excluded from the dataset (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 (1214, 47.1%), OAD-M (94, 3.7%), OAD-P (700, 27.2%), OAD-E (302, 11.8%).

3.3.3 Breed information

A data set with breed composition (expressed in sixteenths) for each cow was used to classify the cows into three breed categories; F, J, and crossbred of F×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 ≥ 14/16 or J ≥ 14/16, respectively. Remaining cows were classified as crossbred of F×J. After the breed classification, one OAD and eight TAD milking herds (1,688 cows) were removed from previously selected herds as they did not have any F, J or F×J cows.

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

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

3.3.4 Parity

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

3.3.5 Reproduction data set

Cows that did not calve between 1st June 2017 and 31st December 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 2562 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 prior to 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 2562 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×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 five and above (232,290; 28.6%).

3.3.6 Fertility traits

The 12 fertility traits investigated in the present study are defined in Table 3.1.

Table 3.1. Description of fertility traits and target values of New Zealand dairy industry and national averages reported in production year of 2017-2018

Trait	Criteria for trait calculation[1]	Target[2] (%)	National average[2] (%)
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	

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

[2] Source: Burke and Fowler (2007).

[3] Source: LIC and DairyNZ (2020).

In the calculation of fertility traits, mating start date was defined as the first of two consecutive days with at least one AB insemination or natural service, where at least 3 of the next 6 days also had mating records. The planned start of calving 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 3.1 details the calculation of date of conception, 21 d pregnancy outcome (PR21), 42 d pregnancy outcome (PR42) and end-of-mating pregnancy outcome (NIC, not in-calf).

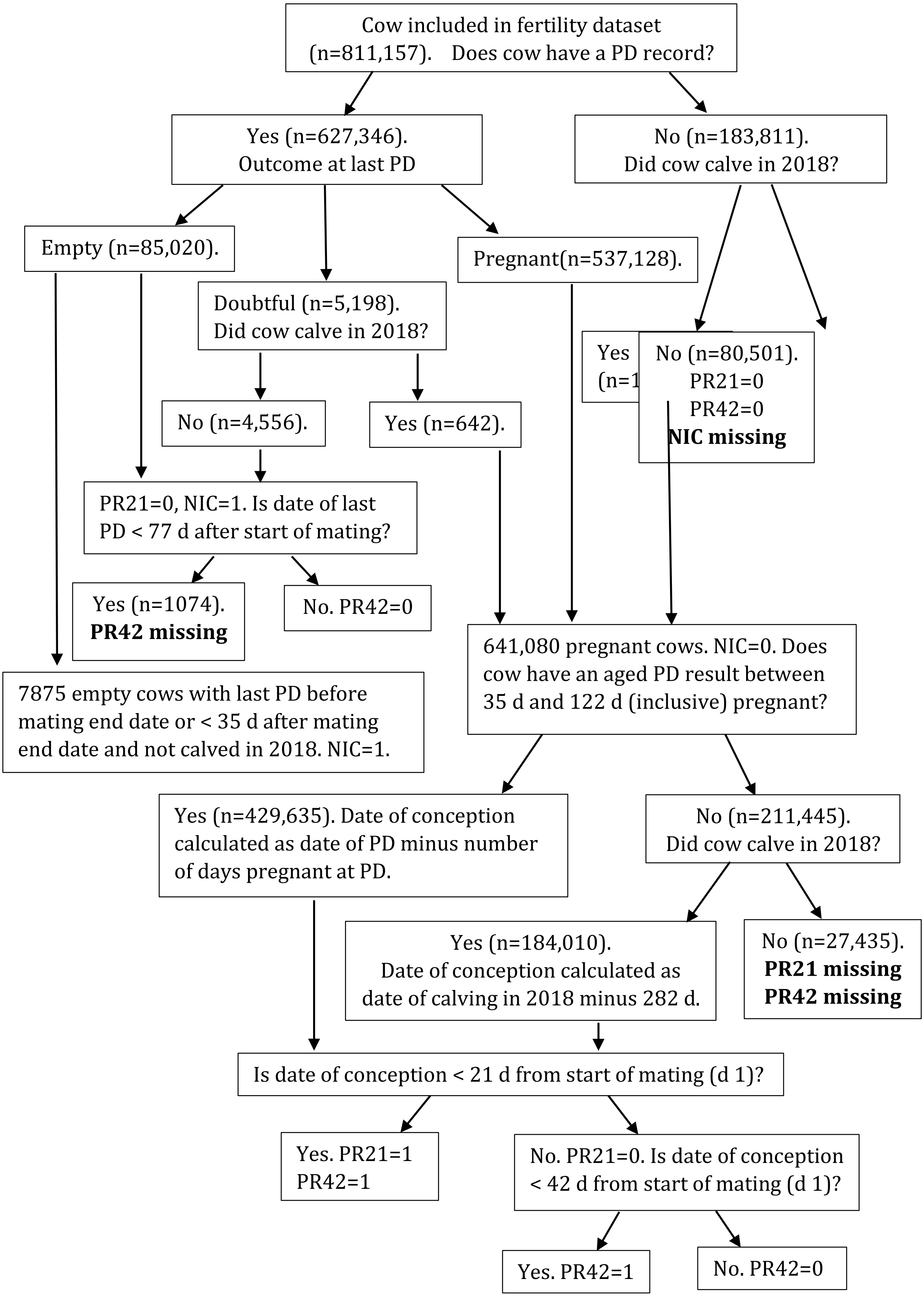

Figure 3.1. Flowchart detailing determination of date of conception, 21 d pregnancy outcome (PR21), 42 d pregnancy outcome (PR42) and end-of-mating pregnancy outcome (NIC, not in-calf), PD= pregnancy diagnosis.

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 d or 42 d of mating (SR21 or SR42) was calculated for all cows in the fertility dataset and coded as 0 for cows that did not have an AB record in the first 21 d 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 equalled 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 d or 42 d after the PSC in 2018, cow was recorded as calved in 21 d (CR21) or 42 d (CR42), respectively. For cows that calved more than 21 d or 42 d after the PSC in 2018, CR21 or CR42 was recorded as 0.

3.3.7 Statistical analysis

All statistical analyses were undertaken using SAS version 9.4 software (SAS Institute Inc. Cary, NC, USA). Binary fertility traits were analysed 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 (1-June to 31-December 2017) to mating start date as covariates, and the random effects of herd and residual. When analysing 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 services per conception was analysed 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 analyse time to event data. Three interval traits were right-censored: SMFS, SMCO and interval form 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 AB insemination that was not followed by another AB inseminations within 7 days. 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.

3.4 Results

Table 3.2 presents herd characteristics with average milk production and average F and J proportions by milking regimen. Compared to 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 while 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.

Table 3.2. Herd characteristic means (standard deviation) according to milking regimen

Category	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)	3201 (860)	4860 (1368)	3957 (1273)	4110 (1071)	4669 (1230)
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)
[2]Breed proportions					
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)

[1] OAD = 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.

[2]F = Holstein Friesian, J = Jersey.

3.4.1 Milking regimen

Least-square means with 95% CI of each fertility trait for milking regimen and parity classes are presented in Table 3.3. 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 3.4. Compared to 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.

Table 3.3. Least-squares means (95% CI for means) of fertility traits for cows milking different parities under different milking regimens[1]

Characteristic	Traits[2]								
	SC	SR21	SR42	PR21	PR42	PRFS	NIC	CR21	CR42
Milking regimen[3]									
OAD	1.31^{b}	83.66^{a}	92.47^{a}	52.76^{a}	75.08^{a}	63.20^{a}	8.90^{c}	63.05^{a}	87.48^{a}
	(1.29-1.33)	(82.57-84.69)	(91.68-93.18)	(51.31-54.21)	(73.93-76.21)	(61.57-64.80)	(8.26-9.58)	(61.39-64.68)	(86.35-88.53)
TAD	1.39^{a}	78.96^{c}	92.66^{a}	43.86^{b}	67.81^{b}	55.00^{b}	12.67^{a}	57.12^{b}	82.89^{b}
	(1.38-1.40)	(78.36-79.55)	(92.31-92.99)	(43.18-44.54)	(67.18-68.43)	(54.19-55.81)	(12.26-13.08)	(56.30-57.93)	(82.23-83.54)
OAD-M	1.40^{a}	79.40^{bc}	91.73^{a}	43.55^{b}	68.23^{b}	53.97^{b}	11.10^{b}	56.51^{b}	82.73^{b}
	(1.36-1.43)	(77.24-81.40)	(90.28-92.98)	(41.60-45.53)	(66.42-69.99)	(51.60-56.33)	(10.09-12.21)	(54.02-58.96)	(80.65-84.62)
OAD-P	1.38^{a}	80.35^{b}	93.02^{a}	44.69^{b}	68.81^{b}	56.05^{b}	11.29^{b}	56.76^{b}	83.20^{b}
	(1.36-1.39)	(79.61-81.08)	(92.58-93.44)	(43.87-45.52)	(68.07-69.55)	(55.07-57.02)	(10.85-11.75)	(55.74-57.78)	(82.38-83.98)
OAD-E	1.38^{a}	79.80^{bc}	92.86^{a}	44.31^{b}	68.39^{b}	55.77^{b}	12.47^{a}	56.87^{b}	83.44^{b}
	(1.36-1.40)	(78.63-80.93)	(92.18-93.49)	(43.09-45.54)	(67.28-69.49)	(54.31-57.22)	(11.77-13.21)	(55.39-58.34)	(82.25-84.55)
Parity									
1	1.39^{a}	77.74^{d}	91.20^{c}	42.86^{d}	66.98^{d}	54.15^{d}	12.26^{b}	55.10^{c}	81.38^{c}
	(1.38-1.40)	(77.06-78.40)	(90.78-91.60)	(42.21-43.52)	(66.35-67.59)	(53.33-54.97)	(11.87-12.67)	(54.28-55.92)	(80.68-82.06)
2	1.37^{c}	79.87^{b}	92.49^{b}	46.36^{c}	70.94^{c}	57.26^{c}	10.40^{c}	57.50^{b}	83.53^{b}
	(1.36-1.38)	(79.24-80.49)	(92.12-92.84)	(45.68-47.05)	(70.35-71.53)	(56.43-58.09)	(10.05-10.76)	(56.68-58.33)	(82.89-84.16)
3	1.36^{d}	82.56^{a}	93.83^{a}	49.31^{a}	73.42^{a}	59.45^{a}	9.33^{d}	60.15^{a}	85.64^{a}
	(1.35-1.37)	(81.97-83.14)	(93.52-94.13)	(48.60-50.01)	(72.84-74.00)	(58.60-60.30)	(8.99-9.67)	(59.31-60.98)	(85.04-86.22)
4	1.36^{d}	82.90^{a}	93.80^{a}	48.26^{b}	71.97^{b}	58.54^{b}	10.28^{c}	59.86^{a}	85.29^{a}
	(1.35-1.37)	(82.30-83.49)	(93.48-94.11)	(47.54-48.98)	(71.35-72.58)	(57.65-59.42)	(9.91-10.66)	(58.99-60.72)	(84.66-85.90)
≥5	1.38^{b}	79.01^{c}	91.02^{d}	42.39^{d}	64.95^{e}	54.15^{d}	14.35^{a}	57.77^{b}	84.07^{b}
	(1.37-1.39)	(78.38-79.62)	(90.59-91.42)	(41.75-43.04)	(64.33-65.56)	(53.35-54.95)	(13.92-14.80)	(56.96-58.57)	(83.46-84.67)
P-values for interaction[3]									
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 in the same column and factor, are significantly different ($P < 0.05$).

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

[2] SC = 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.

[3] OAD = 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.

[4] Interactions of milking regimen and breed were not significant for any fertility traits (Supplemental File S1) and interactions of milking regimen and parity were significant for all fertility traits except SC and CR42 (Supplemental File S2).

Table 3.4. Odds ratios (95% CI and *P*-values) of binary fertility traits for different milking regimens with reference to twice daily milking (TAD) category

Traits[2]	Milking regimen[1]							
	OAD		OAD-M		OAD-P		OAD-E	
	OR[1] (95% CI)	*P*-value	OR[1] (95% CI)	*P*-value	OR[1] (95% CI)	*P*-value	OR[1] (95% CI)	*P*-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

[1] OAD = 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, interval from calving to mating start date (linear and quadratic effects) as covariates; herd was fitted as a random effect.

[2] 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.

The outcomes of Cox proportional hazard models are shown in Table 3.5. 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 3.5). 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 3.2 and 3.3 respectively. Cows milked OAD had slightly increased probabilities for first service by day of the breeding season than TAD (Figure 3.2) and predicted probabilities associated with conception during the breeding season were considerably increased in OAD than TAD (Figure 3.3).

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 3.5). These findings align with the outcomes of the binary traits analysis of SR21, PR21 and PR42. Submission by 3-wks was significantly higher in cows milked OAD-P compared to TAD. Pregnant by 3-wks and 6-wks was not significantly different between cows milked TAD and OAD-M, OAD-P or OAD-E (Table 3.4). Cows milked OAD-M and OAD-P had significantly lower odds ratios for NIC compared to 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 3.3, 3.4 and 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.

Table 3.5. 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

Category	Traits[1]					
	SMFS		SMCO		FSCO	
	HR[1] (95% CI)	P-value	HR[1] (95% CI)	P-value	HR[1] (95% CI)	P-value
Milking regimen[2]						
TAD						
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						
1						
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
P-values for interaction[3]						
Milking regimen×breed		0.08		0.09		0.31
Milking regimen×parity		<0.001		<0.001		<0.001

[1] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); 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.

[2] OAD = 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.

[3] Interactions 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).

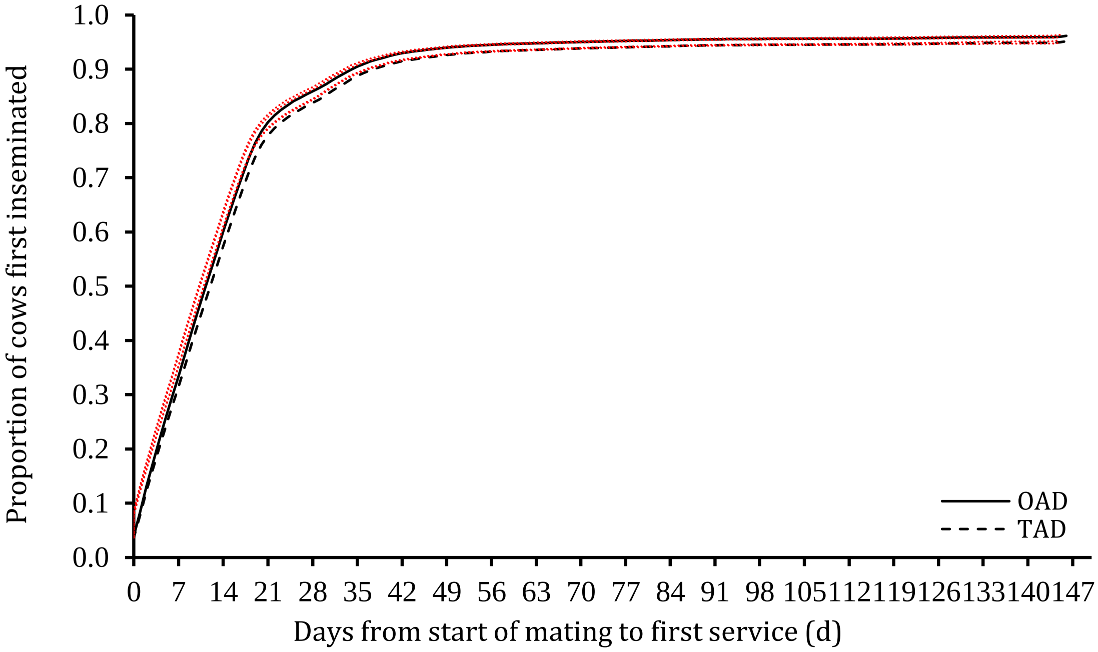

Figure 3.2. Predicted proportions of cows first inseminated by day from start of the mating period from the Cox proportional hazard model in cows milking under once daily for the entire lactation (OAD, solid line) and twice daily for the entire lactation (TAD, dashed line) with 95% CI (red color dotted lines). Predictions were adjusted for proportion of Holstein Friesian, heterosis of Holstein Friesian×Jersey, parity number, linear and quadratic effects of interval from calving to mating start date and herd was fitted as a shared frailty.

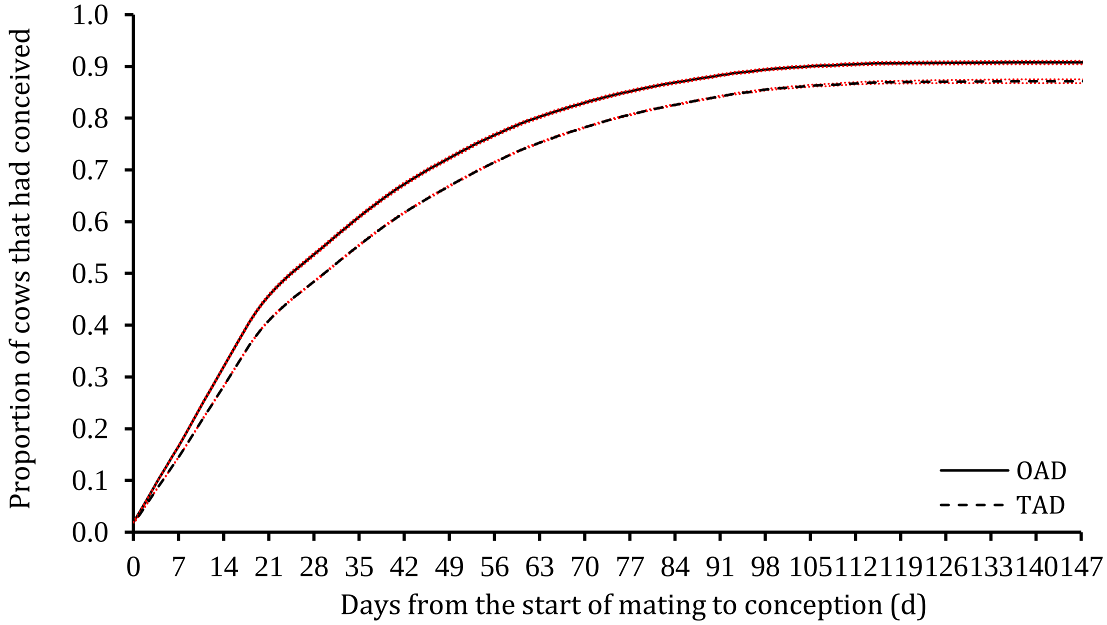

Figure 3.3. Predicted proportions of cows that had conceived by day from start of the mating period from the Cox proportional hazard model in cows milking under once daily for the entire lactation (OAD, solid line) and twice daily for the entire lactation (TAD, dashed line) with 95% CI (red color dotted lines). Predictions were adjusted for proportion of Holstein Friesian, heterosis of Holstein Friesian×Jersey, parity number, linear and quadratic effects of interval from calving to mating start date and herd was fitted as a shared frailty.

3.4.2 Parity

Reproductive performance by parity is presented in Tables 3.3 and 3.5. Estimated hazard ratios for SMFS, SMCO and FSCO were significantly different between first parity cows compared to second, third, fourth and ≥5th parity cows (Table 3.5). Overall reproductive performance as defined by SR21, PR42 and NIC was worst in the oldest (≥5 parity) and youngest (first parity). The general pattern for the binary traits (Table 3.3) was an improvement in fertility from first to third parity, then a plateau in performance in the fourth parity with performance declining from the 5th parity onwards.

3.5 Discussion

In seasonal dairy systems farmers aim to minimise 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 days 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 Britain (range from 37% to 52%) (Royal et al., 2000; Mayne et al., 2002; Sørensen et al., 2008; Norman et al., 2009; Kelleher et al., 2016; O'Sullivan., 2020). We found New Zealand dairy cows had less services per conception (1.39 in 95% CI=1.38-1.40) than cows in Ireland and the United Kingdom (SC from 1.5 to 1.8) (Pryce et al., 1997;

O'Sullivan., 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 impacted by submission rates and conception rates (Xu and Burton, 2003). Despite the relatively high reproductive performance compared to 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 impact 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 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 prior to 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 days after the planned start of calving the more compact calving pattern in OAD herds allows cows more time to recover from calving and resume oestrus activity prior to 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 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 days prior to the start of mating resulted in a higher proportion of cows detected in oestrus compared to TAD milking, but pregnancy rates by 28 days after the start of mating were not significantly different in cows milked OAD or TAD. In contrast, we did find a small positive impact 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 impact 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 challenging 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 (LIC and 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 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 to 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 tests records after end of the mating period were excluded from the calculation of NIC. The absolute values of NIC are, therefore, not comparable between the two 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., 2010; Kay et al., 2012). Phyn et al. (2014) found that cows milked OAD after calving have greater plasma glucose and lower plasma non-esterified fatty acid concentrations than cows milked TAD. Butler (2000) reported NEB during the first three weeks or four weeks 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 to cows milked TAD. Body condition score has been correlated with reproductive performance. Roche et al. (2007) found post calving 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 impact 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 impacts 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 non-pregnant 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.

3.6 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, body condition score and reduced negative energy balance prior to mating. Fertility outcomes were significantly different between different parities. Cows in their 3rd and 4th parities had superior reproductive performance than first parity cows, whilst older cows (parity ≥5) had the poorest reproductive outcomes. The present study indicates the adopting OAD milking for the entire lactation was associated with improved reproductive outcomes.

3.7 References

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3.8 Annexure

Supplemental File S1. Reproductive traits of the number of services per conception (SC), 3-wk submission (SR21), 6-wk submission (SR42), 3-wk in-calf (PR21), 6-wk in-calf (PR42), conception to the first service (PRFS), not in-calf (NIC), 3-wk calving (CR21) and 6-wk calving (CR42) by breed for different milking regimens are presented below respectively. In general, in each milking regimen, F×J cows had the lowest SC, NIC and highest mean percent of SR21, SR42, PR21, PR42, PRFS, CR21 and CR42 than the F and J cows. Friesian, J and their crossbred cows milked under OAD in the entire season had the lowest SC, NIC and highest mean percent of SR21, PR21, PR42, PRFS, CR21, CR42 compared to the F, J and F×J cows milked under TAD and OAD milking in part of the lactation.

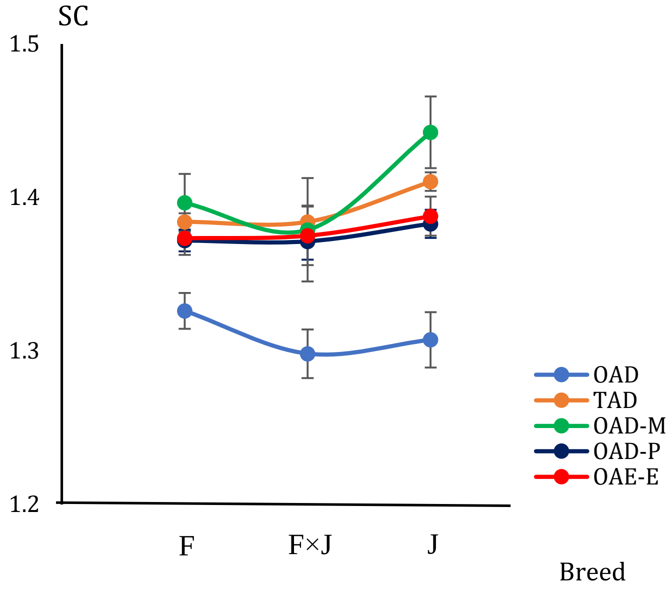


Figure S1.3.1. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on number of services for conception (SC) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

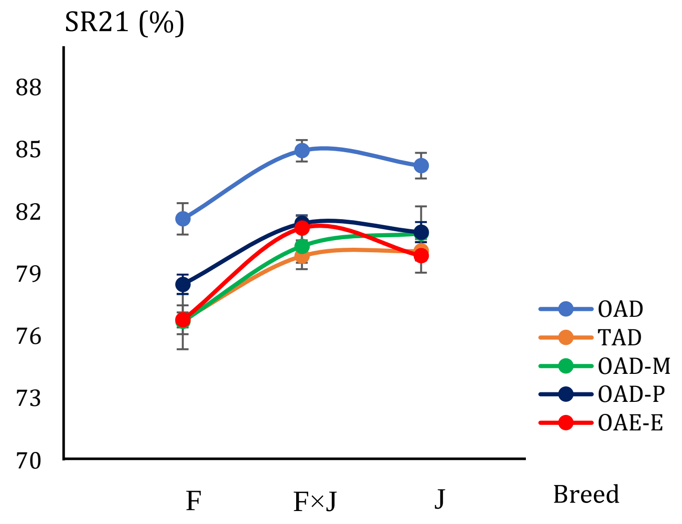


Figure S1.3.2. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 3-wk submission (SR21) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

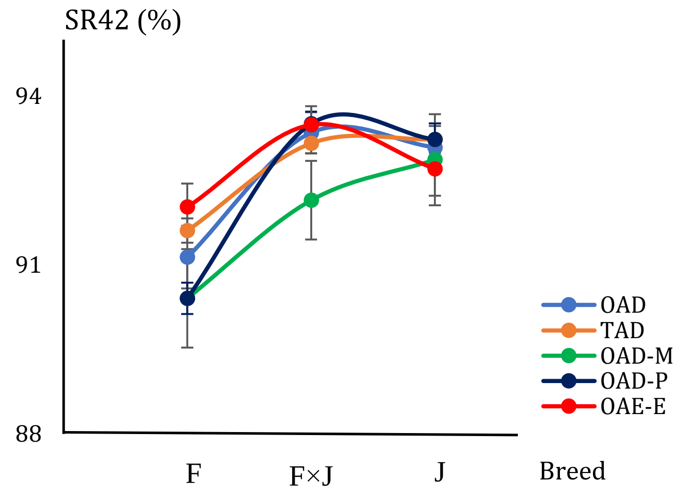


Figure S1.3.3. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 6-wk submission (SR42) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

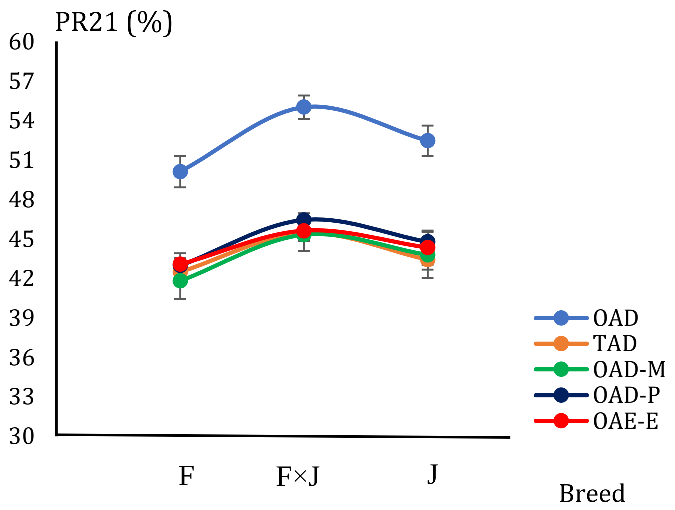


Figure S1.3.4. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 3-wk in calf (PR21) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

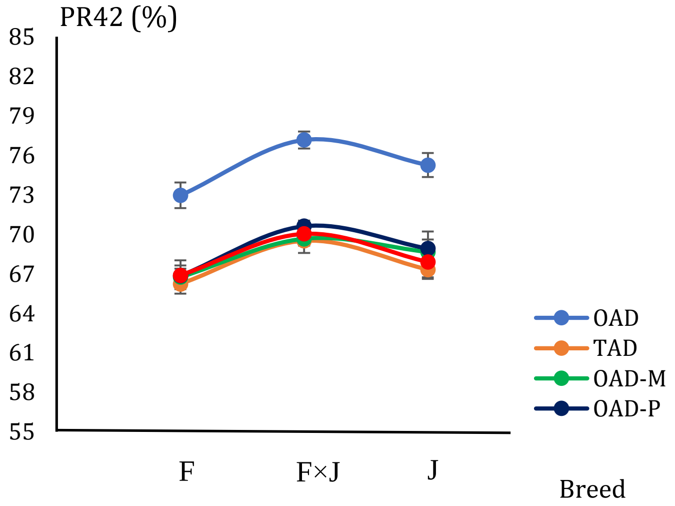


Figure S1.3.5. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 6-wk in calf (PR42) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

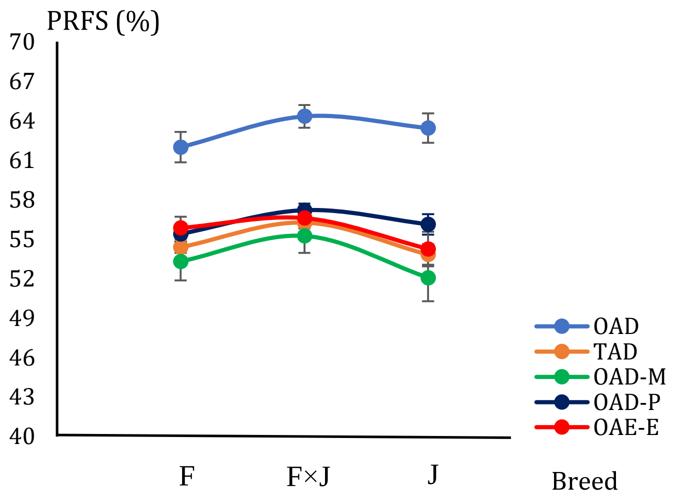


Figure S1.3.6. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on conception to the first service (PRFS) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

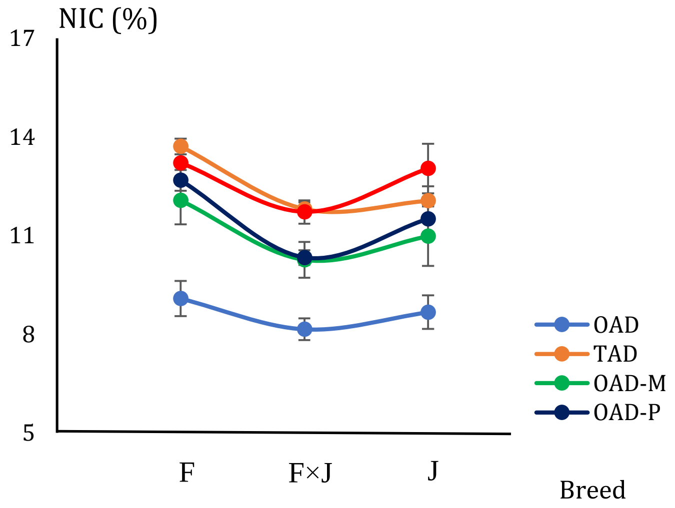


Figure S1.3.7. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on not in calf (NIC) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

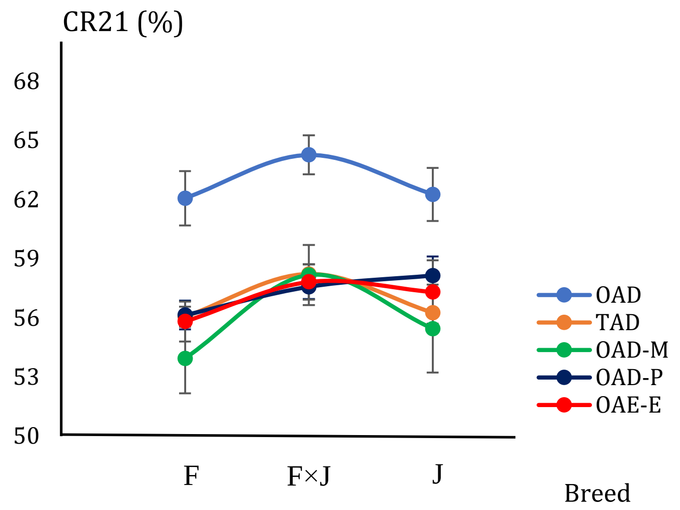


Figure S1.3.8. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 3-wk calving (CR21) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

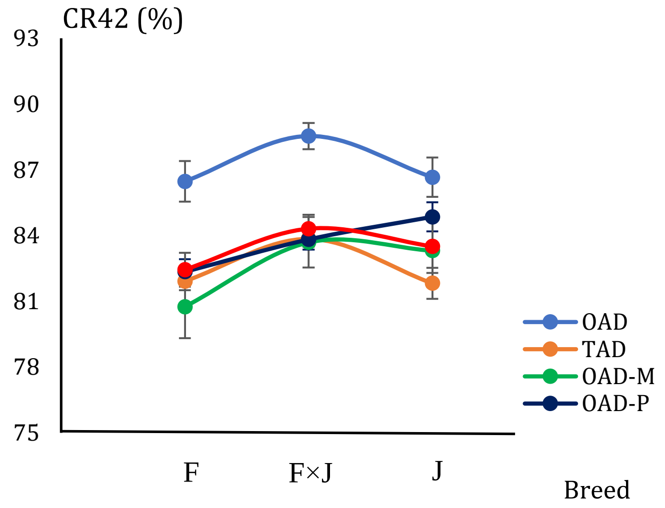


Figure S1.3.9. Interaction effect of milking regimen and breed of Holstein Friesian (F), Jersey (J), crossbred of Holstein Friesian and Jersey (F×J) on 6-wk calving (CR42) in cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

Supplemental File S2. Reproductive traits of the number of services per conception (SC), 3-wk submission (SR21), 6-wk submission (SR42), 3-wk in-calf (PR21), 6-wk in-calf (PR42), conception to the first service (PRFS), not in-calf (NIC), 3-wk calving (CR21) and 6-wk calving (CR42) by parity for different milking regimens are represented in below respectively. Each parity, cows milked under OAD in the entire season had the lowest SC and NIC than cows milked under TAD and OAD milking in part of the lactation. Also, in every lactation cows milked under OAD had a higher mean percent of SR21, PR21, PR42 and PRFS than other milking practices. In general, in each milking system, SC and NIC were decreased from parity 1 to 3 or 4 and then after it was increased, but SR21, SR42, PR21, PR42 and PRFS had an upward trend from parity 1 to 3, steady in 3rd and 4th parities and had downward trend from 4 to parity 5≥.

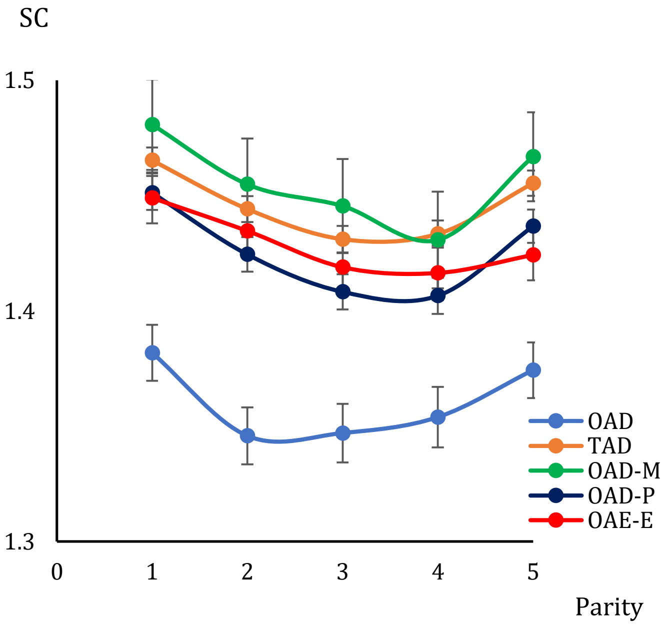


Figure S2.3.1. Interaction effect of milking regimen and parity on number of services for conception (SC) of cows milked OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

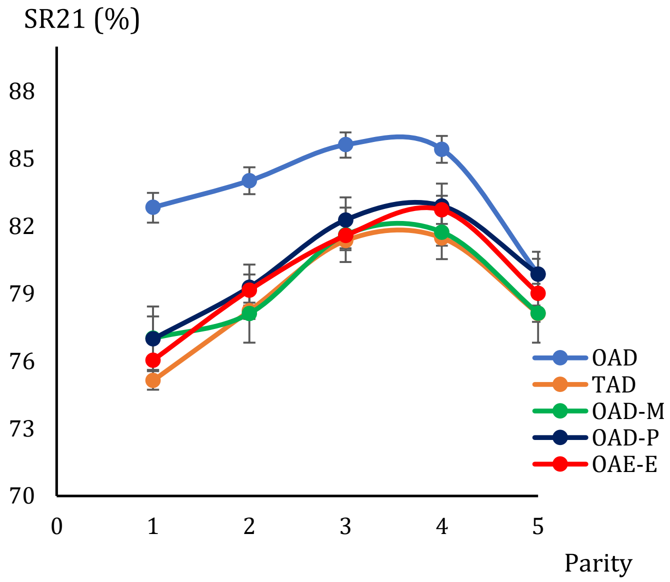


Figure S2.3.2. Interaction effect of milking regimen and parity on 3-wk submission (SR21) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

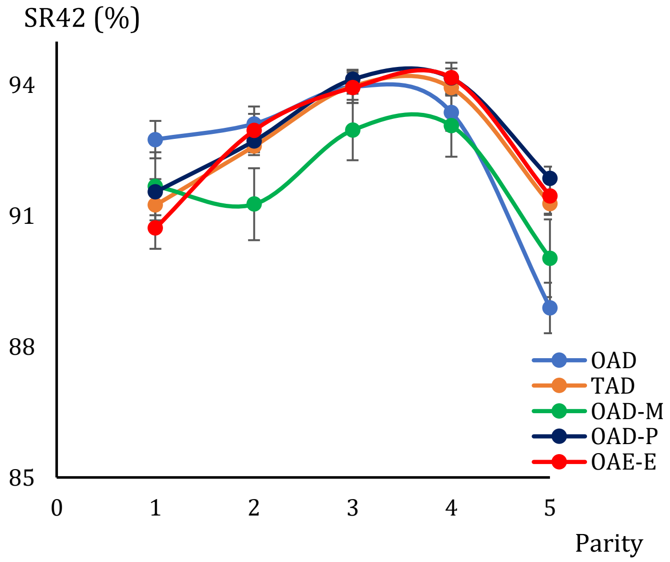


Figure S2.3.3. Interaction effect of milking regimen and parity on 6-wk submission (SR42) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

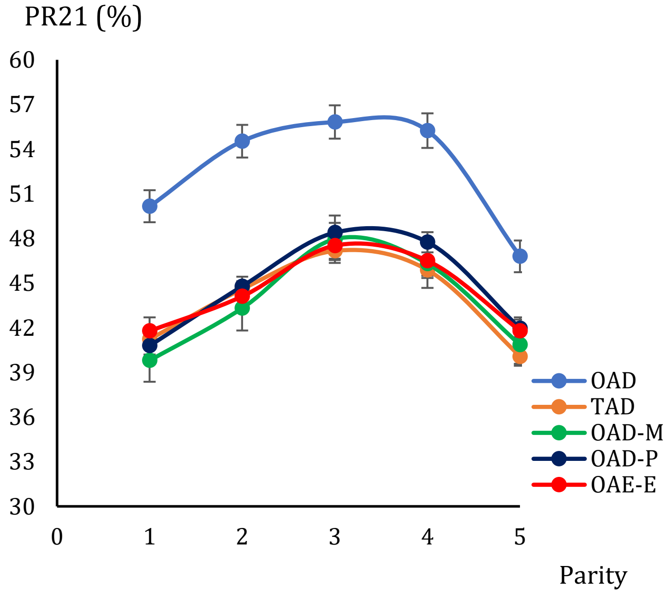


Figure S2.3.4. Interaction effect of milking regimen and parity on 3-wk in calf (PR21) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

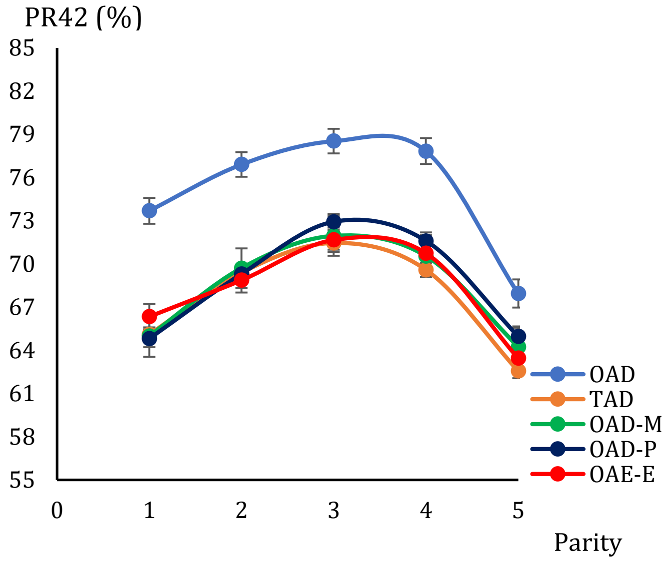


Figure S2.3.5. Interaction effect of milking regimen and parity on 6-wk in-calf (PR42) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

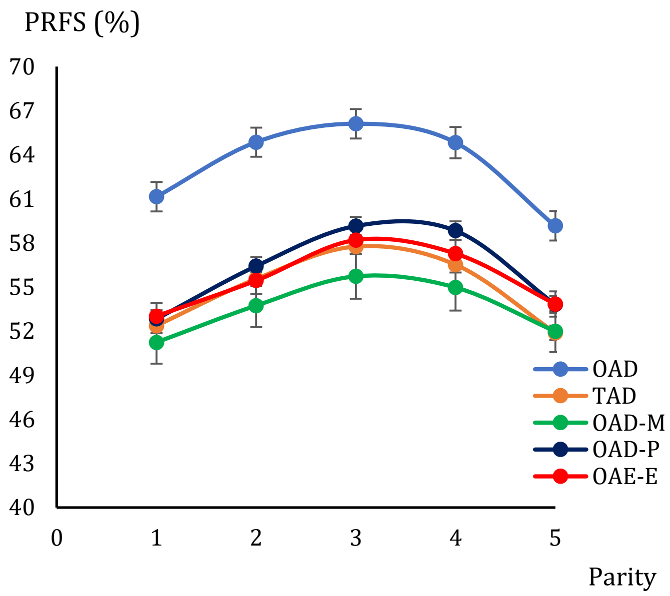


Figure S2.3.6. Interaction effect of milking regimen and parity on conception to first service (PRFS) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

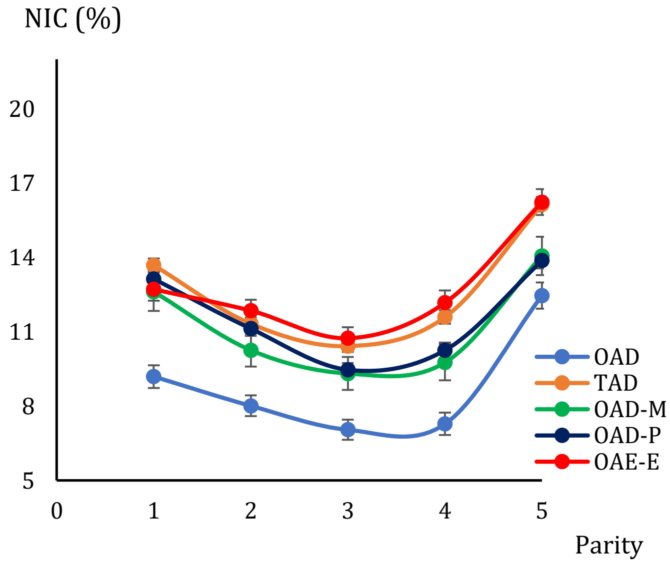


Figure S2.3.7. Interaction effect of milking regimen and parity on not in-calf (NIC) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

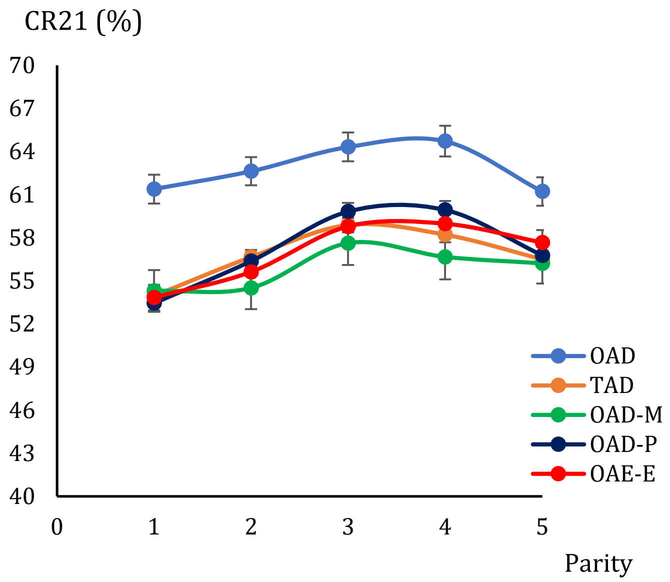


Figure S2.3.8. Interaction effect of milking regimen and parity on 3-wk calving (CR21) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

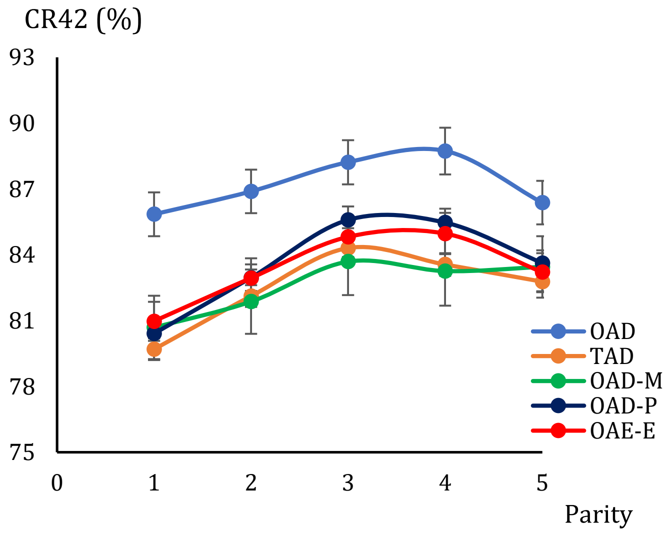


Figure S2.3.9. Interaction effect of milking regimen and parity on 6-wk calving (CR42) of cows milked in OAD once daily, TAD twice daily, OAD-M once daily in cow's mating period, OAD-P once daily after the peak lactation period and OAD-E once daily at end of the lactation.

Supplemental File S3. Hazard ratios (95% CI) for time from start of mating to first service (SMFS), start of mating to conception (SMCO) and first service to conception (FSCO) by parity for different milking regimens of cows milked once daily (OAD), twice daily (TAD), once daily in cow's mating period (OAD-M), once daily after the peak lactation period (OAD-P) and once daily at end of the lactation (OAD-E) are presented below respectively.

Table S3. Hazard ratios (95% CI) of the fertility traits with milking regimens and parity

Traits	Parity	OAD	TAD	OAD-M	OAD-P	OAD-E
SMFS	1					
	2	1.03 (1.01-1.06)	1.05 (1.04-1.06)	1.00 (0.97-1.04)	1.03 (1.01-1.05)	1.07 (1.05-1.10)
	3	1.08 (1.05-1.11)	1.08 (1.07-1.09)	1.04 (1.00-1.08)	1.06 (1.05-1.08)	1.07 (1.05-1.10)
	4	1.05 (1.02-1.08)	1.05 (1.03-1.06)	1.04 (1.00-1.09)	1.05 (1.03-1.07)	1.08 (1.06-1.10)
	5	0.87 (0.85-0.89)	0.93 (0.92-0.94)	0.92 (0.89-0.96)	0.95 (0.94-0.97)	0.94 (0.92-0.96)
SMCO	1					
	2	1.10 (1.07-1.14)	1.10 (1.09-1.11)	1.11 (1.07-1.15)	1.11 (1.09-1.13)	1.09 (1.07-1.12)
	3	1.15 (1.12-1.19)	1.15 (1.13-1.16)	1.15 (1.10-1.19)	1.19 (1.17-1.21)	1.15 (1.13-1.18)
	4	1.10 (1.07-1.14)	1.08 (1.07-1.09)	1.09 (1.05-1.14)	1.14 (1.12-1.16)	1.11 (1.08-1.14)
	5	0.85 (0.83-0.87)	0.88 (0.87-0.89)	0.91 (0.88-0.94)	0.94 (0.93-0.96)	0.91 (0.89-0.93)
FSCO	1					
	2	1.11 (1.08-1.14)	1.09 (1.08-1.11)	1.10 (1.06-1.14)	1.11 (1.10-1.13)	1.08 (1.06-1.10)
	3	1.15 (1.11-1.18)	1.14 (1.12-1.15)	1.14 (1.09-1.19)	1.20 (1.18-1.22)	1.13 (1.11-1.16)
	4	1.11 (1.08-1.15)	1.08 (1.06-1.09)	1.08 (1.03-1.13)	1.15 (1.12-1.17)	1.09 (1.07-1.12)
	5	0.89 (0.86-0.91)	0.90 (0.89-0.91)	0.92 (0.89-0.96)	0.96 (0.95-0.98)	0.92 (0.90-0.94)

Chapter 4

Estimation of genetic parameters and individual and maternal breed, heterosis and recombination loss effects for production and fertility traits of spring-calved once daily or twice daily milking cows in New Zealand

This chapter has been published in

Jayawardana JMDR, Lopez-Villalobos N, Hickson RE and McNaughton LR. 2023. Estimation of genetic parameters and individual and maternal breed, heterosis, and recombination loss effects for production and fertility traits of spring-calved cows milked once daily or twice daily in New Zealand. Journal of Dairy Science. 106:364-380. https://doi.org/10.3168/jds.2022-22053.

4.1 Abstract

The objectives of this study were to estimate genetic parameters and individual and maternal breed, heterosis and recombination loss effects for milk production and fertility traits of Holstein Friesian (F), Jersey (J), and crossbred of Holstein Friesian and Jersey (F×J) cows milked once daily (OAD) or twice daily (TAD) in New Zealand. Data on 278,776 lactations from 30,217 OAD and 170,680 TAD milking cows across 644 spring calving herds were available. Genetic parameters and individual and maternal breed, heterosis and recombination loss estimates were obtained from univariate animal models. Heritability and repeatability estimates for milk production, milk composition, and fertility traits were consistent across the milking frequencies. Heritability estimates for yields of milk, fat, protein and lactose varied between 0.21 and 0.29 in OAD and TAD. Heritability estimates for fertility traits ranged from 0.01 to 0.08 in both populations and estimates were slightly greater in the TAD than OAD milking cows. In both milking populations, individual breed effects for yields were in favour of F cows, however, maternal breed effects for yields were in favour of J dams. Jersey cows were more fertile than the F cows in both milking populations, but maternal breed effects for fertility traits were in favour of F dams. Individual heterosis effects were favourable for all traits and were consistent across milking regimens. Crossbred F×J cows had a significantly shorter interval from start of mating to first service, start of mating to conception and a higher proportion of 3-wk submission, 3-wk in-calf and 3-wk calving relative to the average of purebred F and J cows. Recombination loss effects were not always unfavourable for production and fertility traits, but most estimates were small with higher standard errors. Favourable maternal heterosis effects were associated with production traits in both milking systems, but maternal heterosis effects were less likely to influence reproductive performance.

Key words: once daily milking, fertility, crossbreeding, heritability

4.2 Introduction

Estimates of genetic parameters for milk production and fertility traits have been reported in seasonal dairy-producing systems (Grosshans et al., 1997; Pryce et al., 1998; Berry et al., 2013), however, most reported genetic parameters for production and fertility traits are associated with data from cows milked twice daily (**TAD**). Since the late 1990s, some farmers in New Zealand have been milking their cows once daily (**OAD**) for the entire lactation (Davis et al., 1999) and about 9% of herds that underwent herd-testing in the production season 2015-2016 were milked OAD for the whole lactation (Edwards, 2018). Some studies reported that cows milked OAD for the entire lactation had better reproductive performance than cows milked TAD for the entire lactation (Clark et al., 2006; Edwards, 2018; Hemming et al., 2018). Estimates of genetic parameters for fertility traits in the OAD milking cow population have not been reported.

Since 1985, crossbreeding has been adopted as the predominant mating strategy in New Zealand (Montgomerie, 2005). At present, the breed composition of the national herd is comprised of crossbred Holstein-Friesian × Jersey cows (**F×J**; 49.6%), Holstein-Friesian (**F**; 32.5%), Jersey (**J**; 8.2%), other breeds and crosses (9.3%), and a small proportion of Ayrshire (0.4%) (LIC and DairyNZ, 2021). In New Zealand, a cow’s lifetime ability to convert feed into farm profit is measured using the Production Worth index (Harris, 2005). The PW index is calculated as the sum of estimated production values for lactation yields of milk, fat and protein, cow mature live weight and somatic cell score each multiplied by the corresponding economic value. The production values are calculated as the sum of estimated breeding value, permanent environment effect and heterosis effect (Harris et al., 2007).

The breed and heterosis effects for milk production traits have been reported in grazing dairy cattle milked with TAD and OAD in New Zealand (Harris and Kolver, 2001; Lembeye et al., 2015), however, the breed, heterosis and recombination loss effects for fertility traits have not been investigated cows milked OAD. Earlier, Ahlborn-Breier and Hohenboken (1991) estimated individual and maternal breed and heterosis effects for yields of milk, fat, and fat percentage for New Zealand dairy cows milked TAD, however, maternal heterosis effects for protein and lactose yield, milk composition and fertility traits have not been estimated. If heterosis effects for the OAD milking population are different from the TAD milking population then there could be bias introduced into the calculation of production values. Therefore, it is important to calculate heterosis effects for the OAD milking population.

This present study had two objectives: firstly, to estimate genetic parameters (variance components, heritability and repeatability) for production and fertility traits in the OAD milking population; secondly, to estimate the individual and maternal breed, heterosis and recombination loss effects for production and fertility traits of OAD and TAD milking cows.

4.3 Materials and methods

4.3.1 Herd selection

Because no live animals were used in this study, institutional animal care and use committee approval was not required. Animal records on herd-test milk yields, calving, mating, pregnancy diagnosis, lactation yields, and ancestry information of dairy cows in three spring calving seasons from 2015-2016 to 2017-2018 in New Zealand were extracted from Livestock Improvement Corporation (LIC) database. The key criteria in herd selection for the present study were: at least 50 cows in the herd, at least four herd tests in a given lactation, pregnancy test results recorded for at least 80% of cows that calved in the 12-month period and "early aged pregnancy testing" (tested on or between 35-122 d of pregnancy) and fetal age estimated for at least 80% of cows in the herd. A total of 2,421 spring calving herds with 1,632,461 cows and 2,704,600 lactations met these criteria.

Herds were classified into two categories, OAD or TAD milking throughout the entire lactation, using the information on herd-test day milking frequency. If more than 90% of the tested cows on a herd test date were milked either OAD or TAD in a herd, then it was classified as OAD or TAD milking herd on that herd test date. All herd tests were classified from 2015-2016 to 2017-2018 seasons. If all the herd tests were classified as OAD throughout the period, then the herd was identified as an OAD milking herd, whereas if all herd tests were classified as TAD, then the herd was identified as a TAD herd. Herds with both OAD and TAD tests were excluded from the analysis. The geographical proximity of herds was determined using GPS Visualizer (Schneider, 2012), and any herds that were more than 20 km from the nearest herd of opposite milking frequency were excluded from the analysis. A total of 668 herds were identified, made up of 114 OAD and 554 TAD herds. Herds where the start of the breeding season occurred before the 15th of September in each spring calving season were removed (n=21) from the data set. Cows and their sire, dam, maternal granddam, and maternal grandsire with more than 12.5% of a breed other than F or J were excluded (n=18,150). Consequently, herds that do not have F, J, and crossbred F×J cows were excluded (3 TAD herds). Finally, 113 OAD and 531 TAD herds with 231,751 cows were selected in this study (Figure 4.1).

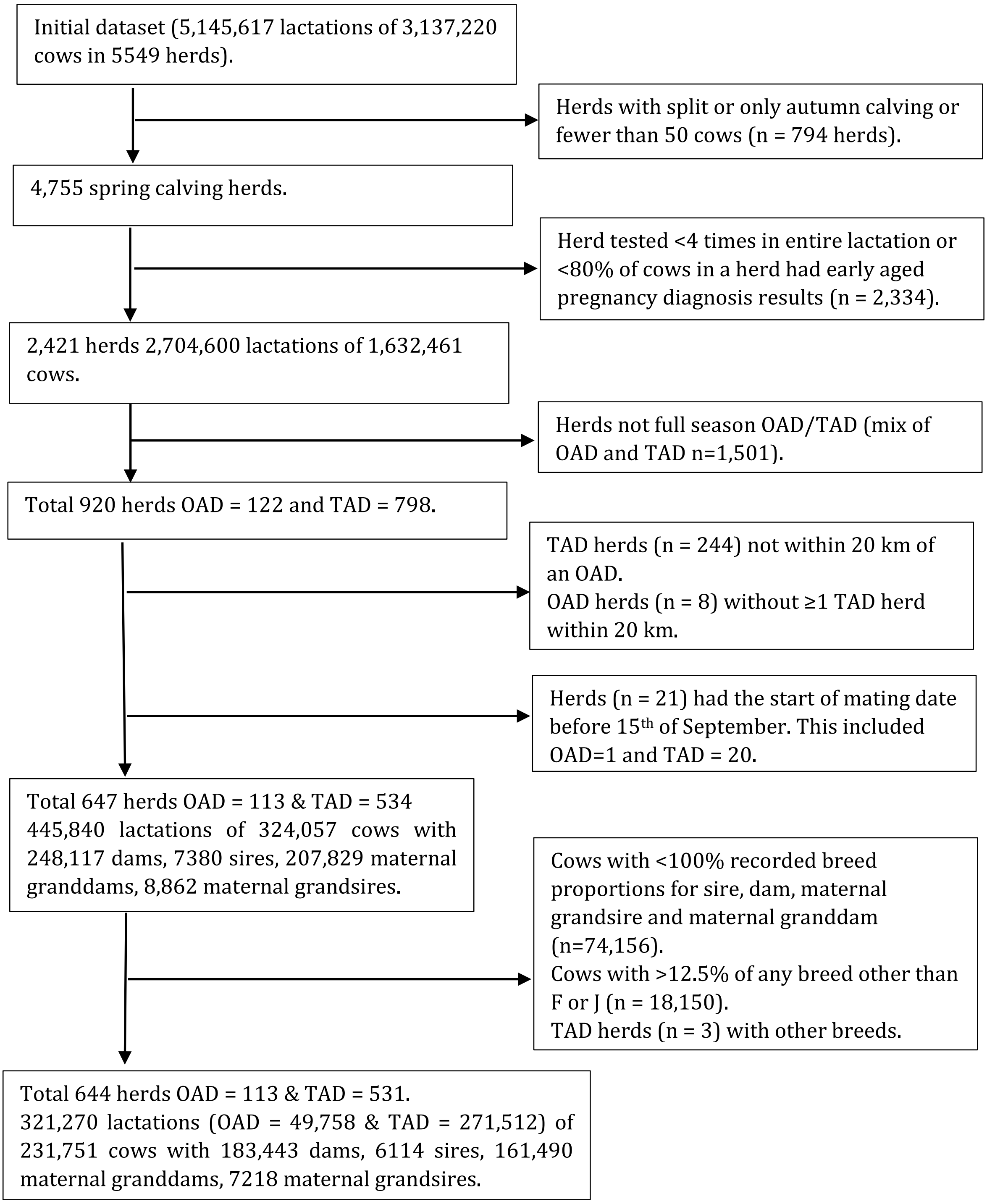


Figure 4.1. Flowchart detailing inclusion of herds that cows milked once daily (OAD) or twice daily (TAD) during the entire lactation.

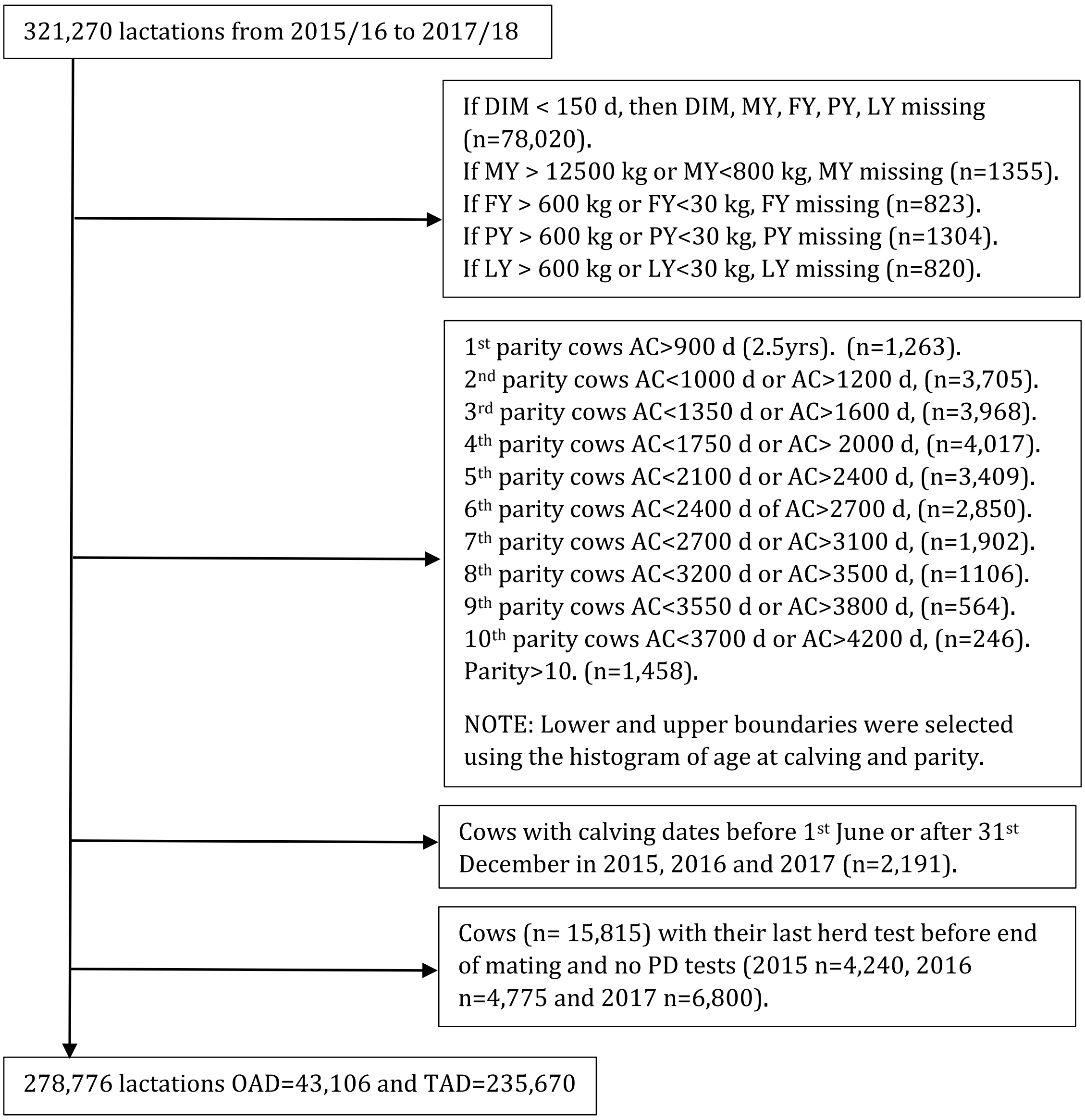
4.3.2 Production data set

Lactation records of milk (**MY**), fat (**FY**), protein (**PY**) and lactose (**LY**) yields (kg) with days in milk ranging from 150 d to 305 d were included (243,250). Lactations with milk yield greater than 12,500 kg or less than 800 kg, or fat, protein, and lactose yields greater than 600 kg or less than 30 kg, were removed (n=4,302) due to uncertainty of the records. The means of MY, FY, PY and LY were 4657 kg, 224 kg, 180 kg, and 234 kg, respectively (Table 4.1). Fat percentage (**FP**) and protein percentage (**PP**) and lactose percentage (**LP**) was calculated for each lactation record as the ratio between FY, PY, or LY and MY multiplied by 100.

Table 4.1. Trait abbreviations, descriptions, means and standard deviations (sd) of traits in selected cow population

Abbreviation	Description	Mean	Sd
Production traits			
MY	305-day milk production (kg)	4657	1441
FY	305-day fat production (kg)	224	64
PY	305-day protein production (kg)	180	52
LY	305-day lactose production (kg)	234	73
FP	Fat percentage, kg fat per kg milk (%)	4.9	0.8
PP	Protein percentage, kg protein per kg milk (%)	3.9	0.3
LP	Lactose percentage, kg lactose per kg milk (%)	5.0	0.2
Fertility traits			
SMFS	Interval from the start of mating to the first service (d)	12.1	10.2
SMCO	Interval from the start of mating to conception (d)	24.3	19.8
FSCO	Interval from the first service to conception (d)	11.9	17.9
CFS	Interval from calving to first service (d)	77.3	19.8
CI	Interval between two consecutive calvings (d)	369.3	24.5
SR21	Presentation (1) or non-presentation (0) for mating within the first 21 days of the start of the mating (binary)	0.82	0.4
SR42	Presentation (1) or non-presentation (0) for mating within the first 42 days of the start of the mating (binary)	0.94	0.2
PR21	Conceived (1) or not conceived (0) within the first 21 days of mating (binary)	0.47	0.5
PR42	Conceived (1) or not conceived (0) within the first 42 days of mating (binary)	0.69	0.5
PRFS	Conceived (1) or not conceived (0) to first service (binary)	0.56	0.5
NIC	Not in-calf (1) or in-calf (0) by end of the mating period (binary)	0.13	0.3
CR21	Calved within the first 21 d of the planned start of calving date of herd in the subsequent season (1) or calved after the first 21 d from the planned start of calving date of herd in the subsequent season (0). Cows that did not calve in the subsequent season were treated as missing variables (binary).	0.58	0.5
CR42	Calved within the first 42 days of the planned start of calving date herd in the subsequent season (1) or calved after the first 42 days from the planned start of calving in the subsequent season (0). Cows that did not calve in the subsequent season were treated as missing variables (binary)	0.83	0.4

Age at calving was calculated for cows in each parity. Cows in first lactation that were greater than 900 d of age at calving were excluded (n=1,263; 1.7%). Age at second, third, and fourth calving was restricted to be between 1000 d and 1200 d, 1350 d and 1600 d, and 1750 d to 2000 d, respectively. Age restriction for fifth and greater than fifth calvings was detailed in Figure 4.2. Lactation records were grouped into five parity classes, cows with parity numbers 5 to 10 were grouped into parity 5 and cows with more than 10 parities were excluded (n=1,458).



321,270 lactations from 2015/16 to 2017/18

If DIM < 150 d, then DIM, MY, FY, PY, LY missing (n=78,020).
If MY > 12500 kg or MY<800 kg, MY missing (n=1355).
If FY > 600 kg or FY<30 kg, FY missing (n=823).
If PY > 600 kg or PY<30 kg, PY missing (n=1304).
If LY > 600 kg or LY<30 kg, LY missing (n=820).

1st parity cows AC>900 d (2.5yrs). (n=1,263).
2nd parity cows AC<1000 d or AC>1200 d, (n=3,705).
3rd parity cows AC<1350 d or AC>1600 d, (n=3,968).
4th parity cows AC<1750 d or AC> 2000 d, (n=4,017).
5th parity cows AC<2100 d or AC>2400 d, (n=3,409).
6th parity cows AC<2400 d of AC>2700 d, (n=2,850).
7th parity cows AC<2700 d or AC>3100 d, (n=1,902).
8th parity cows AC<3200 d or AC>3500 d, (n=1106).
9th parity cows AC<3550 d or AC>3800 d, (n=564).
10th parity cows AC<3700 d or AC>4200 d, (n=246).
Parity>10. (n=1,458).

NOTE: Lower and upper boundaries were selected using the histogram of age at calving and parity.

Cows with calving dates before 1st June or after 31st December in 2015, 2016 and 2017 (n=2,191).

Cows (n= 15,815) with their last herd test before end of mating and no PD tests (2015 n=4,240, 2016 n=4,775 and 2017 n=6,800).

278,776 lactations OAD=43,106 and TAD=235,670

Figure 4.2. Flowchart detailing inclusion of cows in production and reproduction datasets. OAD = once daily milking, TAD = twice daily milking, DIM = days in milk, AC = age at calving, MY= milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, PD = pregnancy diagnosis.

4.3.3 Reproduction data set

Calving dates between 01st June and 31st December in each calving season were retained (n=306,126). Mating start and end dates were sourced from the LIC database. The mating start date of a herd was defined as the first of two consecutive days, both with at least one mating recorded, where at least 3 of the next 6 days also have mating records (DairyNZ, 2019). The mating end date of the herd was defined as either i) the last recorded mating date on or before the 21st wk of the mating period, or ii) the last date with two conceptions followed by at least 30 d with no conceptions on or before the 21st wk of the mating period, or iii) the last date with one conception on or before the 21st wk of the mating period that was followed by at least 30 d with no conceptions and had at least one conception on each of two or more other days in the 6 preceding days (DairyNZ, 2019). The average length of the breeding season was 76.1 d across herds in the study. If any recorded mating dates were outside the herd's mating period, then those mating records were not used for the fertility trait calculations (n=1,034). Cows with the interval from calving to first service (**CFS**) less than 7 d were excluded (n=20). Pregnancy diagnosis testing was conducted using transrectal ultrasound or rectal palpation. Cows that were missing both a pregnancy diagnosis record and all herd tests after the end of the mating period were considered to have left the herd prior to the end of mating and were excluded from the data set (n=15,815).

Following editing of the data as above 43,106 OAD and 235,670 TAD lactations across 644 herds were included in the analysis (Figure 4.2). The data set included 200,897 cows with pedigree over five generations of 160,992 dams, 5,485 sires, 142,383 maternal granddams and 6,588 maternal grandsires. The distribution of cow by breed was 72,279 (36.0%) F, 20,803 (10.4%) J and 107,815 (53.7%) F×J cows and distribution of cows by dam breed was 74,792 (37.2%) F dam, 24,600 (12.3%) J dam and 101,505 (50.5%) F×J dams. The distribution of cows by breed and parity in the OAD and TAD milking is presented in Table 4.2.

Table 4.2. Distribution of cows by breed, parity and production means (standard deviation) according to milking regimen

	Once daily milking	Twice daily milking
Breed		
Holstein Friesian (F)	4,148	68,131
Jersey (J)	7,637	13,166
Crossbred (F×J)	18,432	89,383
Parity		
1	10,648	58,220
2	5,967	31,119
3	3,892	22,625
4	3,219	17,664
≥5	6,491	41,052
Production		
Milk yield (kg)	3,253 (919)	4,929 (1,363)
Fat yield (kg)	170 (45)	234 (62)
Protein yield (kg)	134 (36)	189 (50)
Lactose yield (kg)	161 (46)	248 (69)
Fat percentage (%)	5.31 (2.1)	4.82 (0.7)
Protein percentage (%)	4.15 (3.0)	3.86 (0.3)
Lactose percentage (%)	4.97 (0.2)	5.04 (0.2)

4.3.4 Fertility traits

A range of fertility traits was defined to reflect the features of seasonal calving and breeding in New Zealand dairy cows (Table 4.1). The estimation of the genetic parameters will be biased if the cows with no AB or no conceptions were excluded for time to event variable analysis. Therefore, cows with no recorded artificial breeding (**AB**) inseminations (n=12,602) were retained for the genetic parameter estimation of intervals from start of mating to the first service (**SMFS**) and CFS, and they were assigned the first service date as the end of the AB period in each herd within the calving season. The end of the AB period was defined for each herd as the date of the last AB insemination that was not followed by another AB inseminations within 7 d. Cows that had not conceived (n=42,613) at the end of the mating season were also retained and they received penalty dates for conception. The conception dates were assigned for non-pregnant cows as the mating end date in each herd plus 21 d (Johnston and Bunter, 1996; Grosshans et al., 1997). Consequently, intervals from the start of mating to conception (**SMCO**) and the first service to conception (**FSCO**) included the information of not pregnant cows with the projected penalised conception dates. Further, cows that did not calve the following season, despite a positive pregnancy diagnosis (84,095) were retained for estimation of the genetic parameters for calving interval (**CI**). These cows were assigned a calving date 282 d from the conception dates and CI was calculated as the assigned calving dates minus the calving date in the respective season.

Submission by 3 wk (**SR21**) or 6 wk (**SR42**) of the breeding season was coded as 1 if the first mating date was in the first 21 d or 42 d from the start of mating date, respectively, otherwise coded as 0. The conception to the first service (**PRFS**) was only calculated for cows with a first service date and was coded as 1 if the first service date was equal to the conception date and otherwise 0 (Annexure 1). Cows with recorded mating dates that were outside the mating period were recorded as missing for the calculation of SR21, SR42 and PRFS due to uncertainty of the records. Details for the calculation of conception dates, in-calf by 3-wk (**PR21**), in-calf by 6-wk (**PR42**), and not in-calf (**NIC**) are presented in Figure 4.3. Conception dates were calculated as the date of PD testing minus the estimated pregnancy day count for cows with a pregnancy status of ‘pregnant’. If cows with positive PD had estimated pregnancy day counts outside the bounds (<35 d or >122 d) or no estimated fetal age results, but calved in the subsequent season, their conception dates were calculated as subsequent calving dates minus gestation length of 282 d. Cows were defined as NIC if they were recorded as not pregnant at last pregnancy testing date after the end of the mating period. Planned start of calving (**PSC**) date was derived for a herd by adding 282 d to the herd’s mating start date in each calving season. The calculations of calving by 3 wk (**CR21**) and 6 wk (**CR42**) from the PSC in the subsequent calving season are presented in Figure 4.4.

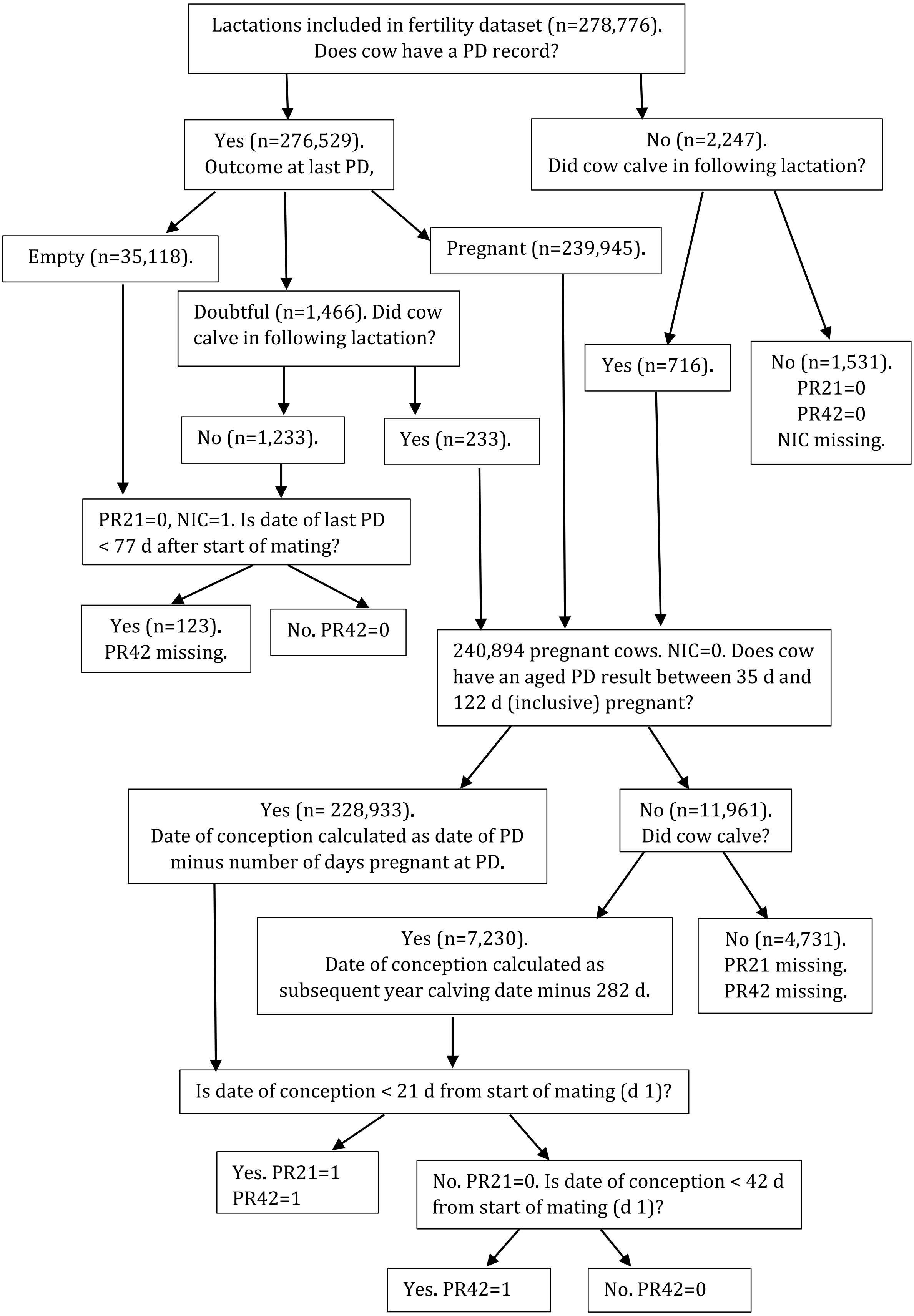


Figure 4.3. Flowchart detailing determination of 3-wk in-calf (PR21), 6-wk in-calf (PR42) and not in-calf at end of the mating period (NIC) after pregnancy diagnosis (PD).

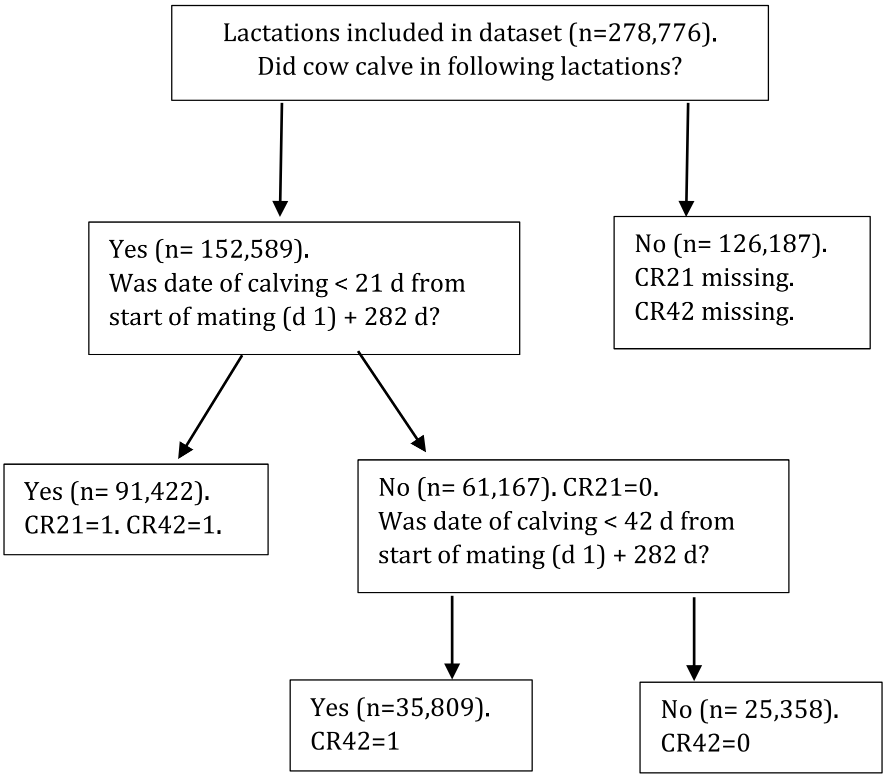


Figure 4.4. Flowchart detailing determination of 3-wk calving (CR21) and 6-wk calving (CR42).

The summary statistics for the fertility traits in the selected population are shown in Table 4.1. The proportion of cows mated within the first 3 wk and 6 wk of the breeding season was 82% and 94%, respectively. Fifty-six percent of the cows were pregnant to their first service and 47% and 69% of cows conceived by the first 3 wk and 6 wk of the breeding season, respectively. Only 13% of cows in the dataset were not in-calf at the end of breeding seasons. Average calving rates by 3 wk and 6 wk were 58% and 83%, respectively. Reproductive performances by milking regimens in OAD and TAD were: 86.5% and 80.8% for SR21, 77.6% and 67.7% for PR42, 62.6% and 54.5% for PRFS, 63.9% and 57.0% for CR21, 87.2% and 81.6% for CR42 and 9.4% and 13.9% for NIC, respectively.

4.3.5 Individual and maternal heterosis and recombination loss coefficients

The coefficients for individual and maternal heterosis and recombination loss were calculated using the animal ancestral information with 16th breed proportions. The individual and maternal heterosis coefficients ($h_{F\times J}$) were calculated using the following equation (Dickerson, 1973):

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

When calculating the individual heterosis coefficients for progeny, α_F^S and α_J^S represent breed proportions of F and J in the sire, respectively; and α_J^D and α_F^D represent breed proportions of J and F in the dam, respectively. When calculating the maternal heterosis coefficients of the dam, α_F^S and α_J^S represent breed proportions of F and J in the maternal grandsire, respectively; α_J^D and α_F^D represent breed proportions of J and F in the maternal granddam, respectively.

Individual and maternal recombination loss coefficients (r) were calculated using following equation (VanRaden and Sanders, 2003):

$$r = 1 - \sum_{i=1}^{2} \frac{sire_i^2 + dam_i^2}{2}$$

where $sire_i$ and dam_i are the proportion of breed *i* in the sire and dam, respectively. In calculation of maternal recombination loss coefficients $sire_i$ and dam_i are the proportion of breed *i* in the maternal grandsire and maternal granddam.

4.3.6 Statistical analysis

Contemporary groups were defined as the group of cows calving in the same herd and year. Estimation of the variance components, heritability, repeatability, individual and maternal breed and heterosis effects was done using ASReml 4.1 software package (Gilmour et al., 2015). Variance components for animal additive genetic variance (σ_a^2), cow permanent environment variance (σ_c^2), random residual variance (σ_e^2) for each production and fertility trait were obtained using the following single-trait repeatability animal model.

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wp} + \mathbf{e}$$

Where **y** is the vector of observations for each production and fertility trait; **b** is the vector of fixed effects, **a** is the vector of animal additive genetic effects; **p** is the vector of random cow permanent environment effects; and **e** is the vector of random residual effects, $\mathbf{X}, \mathbf{Z}, \mathbf{W}$ are incidence matrices relating the phenotypic records to the fixed, animal additive genetic and cow permanent environment effects, respectively.

The fixed effects included in the model were: contemporary group, parity (1,2,3,4, ≥5), the regression coefficients associated with the linear effect of the proportion of F in the cow and dam, the regression coefficients associated with individual and maternal heterosis effects, the regression coefficients associated with individual and maternal recombination loss and the regression coefficients associated with linear and quadratic effects of deviation of calving date from median calving date of the herd within season, respectively.

Variance components related to binary fertility responses in Table 4.1 (SR21, SR42, PR21, PR42, PRFS, NIC, CR21, and CR42) were analysed in a logit scale with the random residual variance of $\pi^2/3 = 3.2899$. Final results were subjected to back-transformation on to linear scale for the interpretation of individual and maternal breed and heterosis effects.

The estimate of heritability (h^2) for each trait was calculated as the proportion between the animal additive genetic and phenotypic variances as follows (Falconer and Mackay, 1996),

$$h^2 = \sigma_a^2/\sigma_p^2$$

Repeatability (t) of each trait was calculated as,

$$t = (\sigma_a^2 + \sigma_c^2)/\sigma_p^2$$

where σ_a^2 is the animal additive genetic variance, σ_c^2 is cow permanent environment variance and σ_p^2 is the phenotypic variance for any trait calculated as $\sigma_p^2 = \sigma_a^2 + \sigma_c^2 + \sigma_e^2$.

4.4 Results

4.4.1 Variance components, heritability, and repeatability

Estimates of variance components, heritability, repeatability, and their standard errors for production and fertility traits for cows in OAD and TAD populations are presented in Table 4.3 and Table 4.4, respectively.

Table 4.3. Estimates of variance components, heritabilities and repeatabilities for milk production and fertility traits for once daily milking (OAD) cow population

Traits	Variance components[1]				Heritability (SE)	Repeatability (SE)
	σ_a^2	σ_c^2	σ_e^2	σ_{Total}^2		
Production[2]						
MY	91,163	92,306	153,940	337,409	0.270(0.015)	0.544(0.007)
FY	223	239	507	969	0.231(0.014)	0.477(0.007)
PY	120	150	268	538	0.223(0.014)	0.502(0.007)
LY	244	248	404	896	0.273(0.015)	0.549(0.007)
FP	0.20	0.06	0.08	0.35	0.580(0.016)	0.764(0.004)
PP	0.04	0.01	0.01	0.06	0.684(0.015)	0.835(0.003)
LP	0.01	0.01	0.01	0.03	0.359(0.016)	0.618(0.006)
Fertility[3]						
SMFS	2.38	2.58	102.25	107.21	0.022(0.005)	0.046(0.007)
SMCO	11.86	9.18	686.57	707.68	0.017(0.004)	0.030(0.007)
FSCO	3.90	2.87	419.15	425.93	0.009(0.003)	0.016(0.006)
CFS	2.38	2.58	102.25	107.21	0.022(0.005)	0.046(0.007)
CI	3.32	2.66	300.33	306.31	0.011(0.003)	0.020(0.006)
SR21	0.20	0.19	3.29	3.68	0.055(0.011)	0.107(0.011)
SR42	0.30	0.26	3.29	3.84	0.077(0.021)	0.144(0.019)
PR21	0.04	0.06	3.29	3.39	0.012(0.004)	0.029(0.006)
PR42	0.08	0.08	3.29	3.45	0.023(0.006)	0.046(0.008)
PRFS	0.03	0.06	3.29	3.38	0.007(0.004)ns	0.026(0.007)
NIC	0.13	0.09	3.29	3.50	0.036(0.012)	0.061(0.015)
CR21	0.06	0.04	3.29	3.40	0.019(0.007)	0.032(0.008)
CR42	0.07	0.07	3.29	3.43	0.021(0.012)ns	0.042(0.015)

[1] σ_a^2= animal additive genetic variance; σ_c^2= cow permanent environmental variance; σ_e^2= random residual variance; σ_{Total}^2= total of phenotypic variance.

[2] MY = milk yield (kg); FY = fat yield (kg); PY= protein yield (kg); LY = lactose yield (kg); FP = fat percentage; PP = protein percentage; LP = lactose percentage.

[3] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); SR21 = cows inseminated in the first 21 days from the start of mating; SR42 = cows inseminated in the first 42 days from the start of mating; PR21 = cows conceived in the first 21 days from the start of mating; PR42 = cows conceived in the first 42 days from the start of mating; PRFS = cows that conceived to their first service; NIC = cows that not in-calf at end of the breeding season; CR21 = cows that calved in the first 21 days from the planned start of the calving; CR42 = cows that calved in the first 42 days from the planned start of the calving.

All heritability and repeatability estimates were significantly different from zero ($P<0.001$) except heritability estimates of PRFS and CR42 (ns defines non-significant estimates).

Table 4.4. Estimates of variance components, heritabilities and repeatabilities for milk production and fertility traits for twice daily milking (TAD) cow population

Traits	Variance components[1]				Heritability (SE)	Repeatability (SE)
	σ_a^2	σ_c^2	σ_e^2	σ_{total}^2		
Production[2]						
MY	198,272	193,621	301,506	693,399	0.286(0.007)	0.565(0.003)
FY	335	450	778	1562	0.214(0.007)	0.502(0.003)
PY	174	253	420	848	0.206(0.007)	0.504(0.003)
LY	502	492	781	1775	0.283(0.007)	0.560(0.003)
FP	0.23	0.06	0.06	0.35	0.656(0.007)	0.814(0.001)
PP	0.04	0.01	0.01	0.06	0.646(0.006)	0.817(0.001)
LP	0.01	0.003	0.02	0.03	0.352(0.007)	0.471(0.004)
Fertility[3]						
SMFS	3.54	12.56	127.28	143.38	0.025(0.003)	0.112(0.004)
SMCO	35.65	33.30	896.01	965.00	0.037(0.003)	0.072(0.005)
FSCO	13.16	18.21	563.42	594.80	0.022(0.002)	0.053(0.005)
CFS	3.55	12.54	127.27	143.35	0.025(0.003)	0.112(0.004)
CI	8.47	0.19	380.98	389.63	0.022(0.002)	0.022(0.004)
SR21	0.26	0.02	3.29	3.56	0.073(0.006)	0.077(0.005)
SR42	0.29	0.006	3.29	3.58	0.080(0.097)	0.081(0.009)
PR21	0.10	1.44E-07	3.29	3.39	0.028(0.002)	0.028(0.002)
PR42	0.12	1.44E-07	3.29	3.41	0.035(0.003)	0.035(0.003)
PRFS	0.06	0.01	3.29	3.36	0.017(0.002)	0.021(0.003)
NIC	0.16	1.37E-07	3.29	3.45	0.046(0.004)	0.049(0.004)
CR21	0.05	1.42E-07	3.29	3.34	0.016(0.002)	0.016(0.002)
CR42	0.08	1.41E-07	3.29	3.37	0.025(0.004)	0.025(0.004)

[1] σ_a^2= animal additive genetic variance; σ_c^2= cow permanent environmental variance; σ_e^2= random residual variance; σ_{Total}^2= total of phenotypic variance.

[2] MY= milk yield (kg); FY = fat yield (kg); PY=protein yield (kg); LY= lactose yield (kg); FP= fat percentage; PP= protein percentage; LP= lactose percentage.

[3] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); SR21 = cows inseminated in the first 21 days from the start of mating; SR42 = cows inseminated in the first 42 days from the start of mating; PR21 = cows conceived in the first 21 days from the start of mating; PR42 = cows conceived in the first 42 days from the start of mating; PRFS = cows that conceived to their first service; NIC = cows that not in-calf at end of the breeding season; CR21 = cows that calved in the first 21 days from the planned start of the calving; CR42 = cows that calved in the first 42 days from the planned start of the calving.

All heritability and repeatability estimates were significantly different from zero ($P<0.001$).

Animal genetic additive, permanent, and residual variances for MY, FY, PY, LY and, interval fertility traits (SMFS, SMCO, FSCO, CFS, CI) were greater in the TAD milking population than the OAD milking population.

In both populations, heritability estimates for yields of milk, fat, protein, and lactose were similar and values ranged from 0.22 to 0.27 in OAD and from 0.21 to 0.29 in TAD. Milk compositional traits (FP, PP, and LP) had greater heritabilities than their respective yields. While heritability estimates for FY and PY are equivalent to LY in both milking systems, FP and PP (0.58 and 0.68 in OAD versus both 0.66 in TAD) are more heritable than LP (0.36 and 0.35 in OAD and TAD, respectively).

Heritability estimates for fertility traits were low (≤0.08) in both populations. Heritabilities for SMFS, SMCO, FCSO, CFS, and CI were low and consistent between OAD (0.01 to 0.02) and TAD populations (0.02 to 0.04). Submission by 3-wk and 6-wk had the greatest heritabilities in both populations. The lowest heritability estimate was for PRFS in OAD (0.01) whereas both PRFS and CR21 had the lowest heritability in TAD (0.02). Heritability estimates for PR21 and PR42 were 0.01 and 0.02 in OAD and 0.03 and 0.04 in TAD, respectively. Heritabilities for CR21 and CR42 were similar across milking frequency (0.02).

Repeatability estimates for yield traits are similar across milking frequency, values ranged from 0.48 to 0.57. Like the heritability estimates, repeatability estimates for milk composition (FP, PP, and LP) were higher (ranging between 0.47 and 0.84) than their respective estimates for yields, and LP had the lowest repeatability compared to FP and PP in OAD and TAD. However, repeatability for LP was higher in the OAD than TAD (0.62 vs 0.47). In both populations, repeatability estimates for fertility traits were low (ranging between 0.02 and 0.14) and similar across milking frequency. The greatest repeatability was estimated for SR42 in OAD (0.14) and SMFS and CFS in TAD (0.11). The repeatability estimates for SMFS and CFS were similar within each population.

4.4.2 Individual breed, heterosis and recombination effects

Table 4.5 shows the estimates of individual and maternal breed and heterosis effects of cows milked in OAD and TAD milking populations. Breed effect is expressed as the difference in performance between F and J breeds.

Table 4.5. Individual and maternal breed, heterosis effects and individual recombination loss coefficients[1] (standard errors) for production and fertility traits of Holstein-Friesian (F), Jersey (J), and crossbred of Holstein-Friesian and Jersey (F×J) cows milked once daily and twice daily

Traits	Once daily milking					Twice daily milking				
	$\mathrm{F}-\mathrm{J}^{I}$	${h_{F\times J}}^{I}$	r^{I}	$\mathrm{F}-\mathrm{J}^{M}$	${h_{F\times J}}^{M}$	$\mathrm{F}-\mathrm{J}^{I}$	${h_{F\times J}}^{I}$	r^{I}	$\mathrm{F}-\mathrm{J}^{M}$	${h_{F\times J}}^{M}$
Production[2]										
MY	869.6(35.5)***	196.7(16.2)***	-23.5(23.1)	-111.3(22.0)*	19.62(12.1)	1397(26.3)***	235.1(11.0)***	-80.9(55.0)***	-108.6(15.8)**	27.3(16.6)***
FY	8.56(1.76)***	12.73(0.81)***	9.57(3.62)***	-2.10(1.11)	-0.57(1.14)	13.22(1.24)***	16.22(0.53)***	9.35(2.56)***	-2.04(0.75)	-1.87(0.78)**
PY	20.42(1.39)**	9.08(0.65)**	6.13(1.36)***	-3.48(0.87)**	1.07(0.48)	31.16(0.90)***	11.04(0.39)***	6.05(1.87)*	-1.89(0.55)	-1.15(0.57)
LY	40.89(1.85)***	10.52(0.85)***	-1.61(1.20)	-5.02(1.15)	1.20(0.63)*	66.4(1.37)***	12.79(0.57)***	-10.35(1.27)***	-4.91(0.82)	0.83(0.86)***
FP	-1.06(0.04)***	-0.01(0.02)	0.25(0.03)***	0.04(0.02)*	0.004(0.01)**	-1.12(0.02)***	-0.02(0.01)	0.14(0.04)***	0.03(0.01)*	-0.05(0.01)***
PP	-0.43(0.02)***	0.0004(0.01)	0.01(0.01)	0.005(0.01)*	0.001(0.01)**	-0.49(0.01)***	0.001(0.003)	0.13(0.02)***	0.03(0.005)	-0.03(0.01)***
LP	-0.08(0.01)***	0.01(0.005)**	-0.02(0.01)	0.02(0.01)***	0.01(0.003)*	-0.06(0.01)***	0.02(0.002)***	0.05(0.01)*	-0.002(0.003)*	-0.01(0.003)*
Fertility[3]										
SMFS	1.16(0.39)***	-0.80(0.25)***	-0.17(0.42)	0.12(0.29)	-0.24(0.18)	1.77(0.25)***	-0.90(0.13)***	-0.34(0.49)**	-0.09(0.17)	0.04(0.16)*
SMCO	1.49(0.97)**	-3.04(0.63)***	-1.60(1.07)	-1.27(0.74)	-0.46(0.46)	2.03(0.67)**	-4.29(0.34)***	-3.25(1.33)*	-0.13(0.44)	0.58(0.44)
FSCO	0.52(0.74)*	-2.10(0.50)***	-0.79(0.85)	-0.96(0.58)	-0.08(0.37)	-0.25(0.51)	-2.81(0.27)***	-2.53(0.98)***	-0.18(0.35)	0.59(0.33)*
CFS	1.16(0.39)***	-0.80(0.25)**	-0.17(0.42)	0.12(0.29)	-0.24(0.18)	1.77(0.25)***	-0.89(0.13)***	-0.31(0.48)**	-0.08(0.17)	0.04(0.16)*
CI	1.14(0.65)*	-1.86(0.44)***	0.56(0.74)	-0.88(0.51)*	-0.02(0.32)	1.74(0.43)***	-1.94(0.23)***	-0.81(0.82)	-0.68(0.29)**	-0.05(0.28)
SR21(%)	-1.97(1.26)*	2.80(0.78)***	-1.11(1.31)	-0.40(0.94)	0.45(0.57)	-6.87(0.79)***	3.08(0.40)***	-1.21(1.56)***	0.93(0.52)*	0.54(0.51)**
SR42(%)	-1.75(0.81)***	1.15(0.51)*	0.15(0.86)*	-0.42(0.61)	1.01(0.37)	-3.75(0.46)***	1.50(0.25)***	-0.60(0.88)**	0.22(0.32)	0.20(0.30)*
PR21(%)	-3.92(1.68)***	5.49(1.13)***	1.65(1.92)	3.55(1.32)**	-0.43(0.83)	-4.59(0.99)***	5.84(0.52)***	1.88(1.95)	1.62(0.67)*	-0.51(0.65)
PR42(%)	-2.87(1.46)***	4.12(0.96)***	2.62(1.63)	2.25(1.13)*	0.68(0.71)	-3.12(0.95)***	5.79(0.49)***	4.46(1.87)*	0.11(0.64)	-1.01(0.62)
PRFS(%)	-3.56(1.61)***	3.15(1.11)***	0.60(1.89)	3.52(1.28)*	-0.44(0.81)	-0.24(0.95)	3.70(0.53)***	1.86(1.78)	1.02(0.67)	-0.54(0.63)
NIC(%)	0.19(1.01)	-2.32(0.68)***	-3.03(1.15)	-0.81(0.79)	-0.62(0.50)	1.69(0.69)*	-3.25(0.36)***	-1.97(1.35)*	0.34(0.47)	0.41(0.45)
CR21(%)	-3.49(2.14)*	2.89(1.45)*	-0.45(2.44)	4.13(1.66)**	-0.59(1.05)	-4.00(1.22)**	4.08(0.72)***	-0.69(2.19)	1.91(0.87)*	0.16(0.80)
CR42(%)	-1.93(1.45)*	1.57(1.00)	2.13(1.70)	3.15(1.14)**	0.88(0.72)	-2.45(0.95)**	2.81(0.57)***	-0.48(1.71)	0.62(0.68)	-0.31(0.63)

*$P<0.05$, ** $P<0.01$ and ***$P<0.001$.

[1] $\mathrm{F}-\mathrm{J}^{I}$= individual breed effect; ${h_{F\times J}}^{I}$ = individual heterosis effect represents the difference between the average of reciprocal first crosses and the average of straightbred cows; r^{I} = individual recombination loss coefficient; $\mathrm{F}-\mathrm{J}^{M}$= maternal breed effect; ${h_{F\times J}}^{M}$= maternal heterosis effect represents the difference between the average of reciprocal first crosses and the average of straightbred dams of cows.

[2] MY= milk yield (kg); FY = fat yield (kg); PY=protein yield (kg); LY= lactose yield (kg); FP= fat percentage; PP= protein percentage; LP= lactose percentage.

[3] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); SR21 = cows inseminated in the first 21 days from the start of mating; SR42 = cows inseminated in the first 42 days from the start of mating; PR21 = cows conceived in the first 21 days from the start of mating; PR42 = cows conceived in the first 42 days from the start of mating; PRFS = cows that conceived to their first service; NIC = cows that not in-calf at end of the breeding season; CR21 = cows that calved in the first 21 days from the planned start of the calving; CR42 = cows that calved in the first 42 days from the planned start of the calving.

For individual breed effects, F cows were superior for milk yields compared to J cows in both milking populations. The superiority of milk production yields in F cows were higher in TAD, which was twice as high as in the OAD milking system, but J cows had greater percentages of fat, protein, and lactose than F cows in both milking systems. The highest individual breed effect was found with MY followed by LY, PY, and FY in both OAD and TAD, respectively. In both milking populations, individual breed effects for fertility traits are in favour of J cows. Results indicate that J cows in OAD milking population had significantly shorter SMFS (<1.2 d), SMCO (<1.5 d), FSCO (<0.5), CFS (<1.2 d), CI (<1.1 d) and significantly higher proportions of SR21 (>2.0%), SR42 (>1.8%), PR21 (>3.9%), PR42 (>2.9%), PRFS (>3.6%), CR21 (>3.5%) and CR42 (>1.9%) than F cows. Likewise, in the TAD population, J cows were superior for fertility traits, having significantly shorter SMFS (<1.8 d), SMCO (<2.3 d), CFS (<1.8 d), CI (<1.7 d), and significantly higher proportions of SR21 (>6.9%), SR42 (>3.8%), PR21 (>4.6%), PR42 (>3.1%), CR21 (>4.0%) and CR42 (>2.5%) than F cows. Estimates for the individual breed effects for SMFS and CFS were similar within the OAD and TAD populations.

Crossbred F×J cows produced significantly higher MY, FY, PY, LY and LP than the average of the parental breeds under both milking regimens. Individual heterosis effects for fertility favoured F×J cows in such a way that they had significantly fewer days from SMFS, SMCO, FSCO, CFS, CI, and significantly higher proportions of SR21, SR42, PR21, PR42, PRFS, CR21, CR42, and a lower proportion of NIC than the averages of parental breeds in both milking systems. In general, individual heterosis effects for fertility traits were similar across the milking populations but heterosis effects for

PR42, CR21 and CR42 tended to be greater in magnitude in the TAD milking population compared with OAD milking population.

Estimates of the individual recombination loss effects were significant and favourable for FY, PY, FP and SR42 in OAD. In the TAD milking population, individual recombination loss effects were significant for all production traits, but estimates were favourable for FY, PY and milk composition traits. Individual recombination loss estimates were significant and unfavourable for SR21 and SR42 in the TAD milking population, although effects were slightly favourable and significant for SMFS, SMCO, FSCO, CFS, PR42 and NIC in the TAD milking population. In general, most of the estimated recombination loss effects were relatively small compared with heterosis effects and recombination effects were less likely to be significant for production and fertility traits in OAD.

4.4.3 Maternal breed, heterosis and recombination effects

The estimates of maternal breed effects for milk production traits were in the opposite direction to the individual maternal breed effects. Maternal breed effects for MY, PY, FP, PP and LP were significant in OAD milking whereas maternal breed effects of MY, FP and LP were significant in TAD milking system. Though individual breed effects for FP, PP and LP were in favour of J cows in both milking populations, maternal breed effects for FP, PP and LP were in favour of F dams. Maternal breed effects were also significant for CI, PR21, PR42, PRFS, CR21 and CR42 in OAD and for CI, SR21, PR21 and CR21 in TAD and these effects were in favour of the F dam.

In both milking populations, maternal heterosis effects for yield traits were similar and maternal heterosis effects for FP, PP and LP were closer to zero. Maternal heterosis effects for LY, FP, PP and LP were significant in OAD. Thus, daughters of crossbred F×J dams had more LY (1.2 kg) and higher FP (0.004%), PP (0.001%) and LP (0.01%) than the average performances of their parental breeds in the OAD milking system. For the TAD milking cow population, estimates of maternal heterosis effects for MY, FY, LY, FP, PP and LP were also significant, daughters of crossbred F×J dams produced 27 kg more MY, 1.9 kg less FY, 0.83 kg more LY and less FP (-0.05 %), PP (-0.03%) and LP (-0.01%) compared to the average performances of purebred F and J dams. In general, maternal heterosis effects for fertility traits were not significant in OAD, however, significant maternal heterosis effects were observed with SMFS, FSCO, CFS, SR21 and SR42 in the TAD milking population. Most of the maternal recombination effects for production and fertility traits were not significant in both milking populations (results not presented).

4.5 Discussion

4.5.1 Production and fertility performances

The means of MY, FY, PY, FP, PP, CI, SR21, PR42 and PRFS were similar to the national averages reported for three seasons from 2015-2016 to 2017-2018 in New Zealand (LIC and DairyNZ, 2021). As reported by Hemming et al. (2018) and Edwards (2018), we found that cows milked OAD for the entire lactation had improved reproductive performance, in terms of the higher mean of SR21, PR42, PRFS, CR42 and lower NIC than cows milked TAD. The improved reproductive performance in OAD milking cows is likely to be due to the improved energy balance in the early lactation of cows milking in OAD compared to TAD (Phyn et al., 2010; Kay et al., 2012; Phyn et al., 2014).

4.5.2 Variance components, heritability, and repeatability estimates

The greater variance components for production traits in the TAD population compared with the OAD population may result from the greater milk production in this population. Nevertheless, the heritability and repeatability estimates for production traits were similar in both milking populations, which could be due to the scale effect.

In general, heritability estimates for yields and milk composition traits are within the range reported in the literature (MY, FY, PY and LY: 0.16 to 0.36; FP, PP and LP: 0.35 to 0.67) in cows milked TAD in New Zealand (Ahlborn and Dempfle, 1992; Bryant et al., 2007; Lembeye et al., 2016; Lopez-Villalobos et al., 2020). Heritabilities of MY, FY, PY, FP and PP in OAD milking population in this study are consistent with the findings of Lembeye et al. (2016) in New Zealand. As far as we are aware this is the first study to estimate genetic parameters for LY and LP in cows milked OAD.

Estimates of heritability for LY were slightly higher than the estimates of heritability for FY and PY in both milking populations. The main export dairy products of New Zealand are whole (WMP) and skim milk powder (SMP), cheese, butter and casein (Sneddon et al. 2016). The standardisation of milk for WMP and SMP requires the three main milk components (fat, protein and lactose) to meet specified codex standards. Sneddon et al. (2016) showed that if the milk from the average New Zealand cow was manufactured without standardisation, the WMP produced is lower in lactose than the required composition. To address this disparity, milk processing companies can purchase lactose to fill the lactose deficit. Sneddon et al. (2016) demonstrated that including LY in the selection index would produce genetic responses towards a milk more suitable for WMP and SMP. Table 4.2 shows that milk produced from OAD milking cows is higher in FP and PP than milk from TAD milking cows. The economic value of selection for lactose in OAD milking cows needs to be estimated to determine whether LY should be included in OAD selection indexes.

The low heritabilities of SMFS, SMCO, FSCO, CFS and CI in both OAD and TAD populations in this study were agree with previously published estimates for TAD populations in New Zealand, British, Irish and Australian dairy cattle populations (ranged between 0.01 and 0.07) (Grosshans et al., 1997; Pryce et al., 1998; Kelleher et al., 2016; Morton et al., 2018). The New Zealand dairy production system depends on a seasonal calving pattern. Measuring fertility using interval traits, such as from calving to the event (CFS and CI) can be biased due differences in the interval from calving to start of the mating season for early versus late calving cows. In New Zealand, the mating season starts on a fixed calendar date, meaning cows that calve early have a longer interval from calving to the start of mating than cows that calve later. For this reason, the two key fertility metrics used in New Zealand are SR21 and PR42. Dairy farmers aim to maintain a 365-d calving interval by minimising the interval between the start of mating and first service and maximising the proportion of cows that conceive within 42 d of the start of mating.

Heritability estimates for SR21 and CR42 have been well reported with New Zealand dairy cattle population (Pryce and Harris, 2006; Bowley et al., 2015; Craig et al., 2018).

However, genetic parameters for SR42, PR21, PR42, PRFS and CR21 are not typically reported. Heritability estimates for SR21 and CR42 in this study are consistent with the previously cited studies in New Zealand (SR21: 0.01 to 0.07 and CR42: 0.01 to 0.029). Heritabilities of PR42 and NIC tended to be slightly higher in TAD than OAD milking cows, but no other studies have reported heritability for NIC in New Zealand for comparison. Morton et al. (2018) reported 0.04 heritability for PR42 and NIC in Australian dairy cattle, similar to the present results. In general, the low heritabilities of fertility traits are a consequence of small genetic variation and relatively large residual variation in both milking systems, which means that management, feeding and environmental factors have a greater influence on reproductive performance than genetic factors.

The similar repeatabilities among OAD and TAD populations for milk yield and milk composition were consistent with previous comparisons of genetic parameters for OAD and TAD production traits in New Zealand (Sneddon et al., 2015a; Lembeye et al., 2016; Lopez-Villalobos et al., 2020). There is a paucity of published estimates of repeatability for fertility traits. The similarities in repeatability of fertility traits between OAD and TAD populations reported here means that inference can likely be made for OAD populations from future studies involving TAD populations.

4.5.3 Individual breed, heterosis and recombination effects

In general, breed effects on milk production agree with published estimates (Ahlborn-Breier and Hohenboken, 1991; Sneddon et al., 2015a; Lembeye et al., 2015). Holstein Friesian cows produced the greatest volumes of milk in either OAD or TAD herds. Breed effects for FY and PY in the OAD milking population were relatively smaller than in the TAD milking population. This is likely due to a scale effect, as cows milked OAD produced less milk than cows milked TAD. Breed effects were negative for percentages of milk components which agree with the findings of Sneddon et al. (2015b), who reported that J cows produced less milk volume but higher concentrations of fat and protein. Breed effects for FP and PP were slightly greater in TAD than in OAD milking and were small for LP in both OAD and TAD. In an experimental study, Clark et al. (2006) found no significant breed and milking frequency interaction for milk compositional traits.

In agreement with the current results, studies in USA (Washburn et al., 2002), Ireland (Prendiville et al., 2011) and Denmark (Freyer et al., 2008) have reported that J cows milked TAD had better reproductive performance than F cows. As was the case in the current study Clark et al. (2006) reported J cows milked in both OAD and TAD had higher SR21 than F cows in New Zealand. McClearn et al. (2020) suggested F herds with poor fertility performance and low milk fat and protein percentages are likely to benefit considerably from crossbreeding with J, and all herds are likely to benefit in terms of production efficiency.

The significant heterosis effects observed for MY, FY and PY for OAD and TAD milking systems in this study agree with the estimates reported by Lembeye et al. (2015). These estimates tended to be greater in TAD than in OAD milking cows. The magnitude of the heterosis effect for first-cross F×J cows in the TAD system in our study are greater than the findings of Harris (2005), who reported heterosis for MY, FY, and PY as 139 kg, 7.7

kg, and 5.5 kg, respectively. A possible reason for this difference is that milk production has increased over time. Average milk, milk fat and protein production per cow in the national herd has increased by 577 kg, 31 kg and 29 kg from production seasons 2004-2005 to 2017-2018 (LIC and DairyNZ, 2021).

In New Zealand, studies by Harris and Kolver (2001) and Harris et al. (2006) reported first-cross F×J cows had 1.5 d shorter SMFS, 10.1% greater pregnancy rate, 4.3% greater SR21 and 3.4% greater CR42 compared to the mean performances of the parental F and J breeds. We found favourable heterosis effects for SMFS, 3-wk and 6-wk pregnancy rates, SR21 and CR42 with F×J cows in both milking systems. However, the magnitude of the effects is slightly lower in the present study compared with previous works. In agreement with our findings, favourable heterosis effects were reported for CFS, CI, SR21 and PR42 in crossbred Holstein × Jersey and Holstein × Friesian cows in Irish and British dairy cattle populations (Wall et al., 2005; Kelleher, 2016) (CFS: -1.1 d; CI: -1.6 d to -9 d; SR21: 4% and PR42: 6%).

Estimated breed effects for fertility traits tended to be greater in TAD than OAD in this study. Although phenotypic reproductive performance is already better in OAD than TAD herds heterosis effects through the use of crossbred cows offer the potential to increase phenotypic performance in both milking systems. Heterosis effects for fertility traits were similar in both milking populations except for 6-wk in-calf and calving rates, indicating positive effects of heterosis on fertility in both milking systems.

There is a paucity of information available in literature for recombination effects of production and fertility traits of crossbred cows in New Zealand. Recombination effects estimated in the present study were small and mix of favourable and unfavourable effects for production and fertility traits. Though significant and favourable recombination effects were observed, the standard errors of these estimates were high. We suggest these estimates should be used with caution when designing breeding programmes using crossbreeding. Further research in this area needs to be conducted. Large negative recombination effects were found MY in the TAD milking population, indicating that much of the F1 heterosis for MY was lost in advanced backcrosses or second crossbred cows. However, recombination effects for FY and PY in both milking populations were positive. This indicates that farmers using crossbreeding systems such as back crossing, 2-breed rotational crosses or the use of crossbred sires will still benefit from favourable heterosis and recombination effects. The use of crossbred sires is a popular breeding strategy in New Zealand. Over 30% of inseminations use semen from crossbred sires (LIC and DairyNZ, 2021). In both milking populations, milk composition traits had relatively small heterosis and recombination loss effects, implying that these traits are more influenced by additive genetic effects rather than interactions between alleles in the same locus or different loci. There is conflicting evidence for the direction of recombination effects in the literature. VanRaden and Sanders (2003) reported slightly favourable recombination for yields in US dairy herds. In contrast Dechow et al. (2007) and Dezetter et al. (2015) reported unfavourable recombination effects for production and fertility traits. In the current study, most recombination effects were not significant in the OAD milking population whereas estimated recombination effects were significant, but small, in the TAD milking population. Heterosis effects have a greater potential to improve production efficiency on-farm than recombination effects.

4.5.4 Maternal breed and heterosis effects

Maternal breed effects in favour of J dams for MY and F dams for FP in both milking populations are consistent with previous research. Ahlborn-Breier and Hohenboken (1991) reported 51 kg of MY in favour of the J dams and 0.1% of FP in favour of F dams in the TAD milking system. Similarly, the low but significant maternal heterosis effect for FP in TAD milking system is similar to the -0.05% reported by Ahlborn-Breier and Hohenboken (1991).

Our results show that maternal heterosis effects for fertility traits were not significant in OAD milking population, suggesting that maternal heterosis has a less impact on cow fertility in OAD milking system. To our knowledge, no previous study has investigated the maternal breed and heterosis effects for fertility traits in New Zealand, therefore, no estimates are available in the present literature to compare our findings. Interestingly, our results indicate daughters of crossbred F×J dams had a significantly higher SR21 and SR42 than the average performance of purebred F and J dams, though they had significantly longer SMFS, FSCO and CFS in TAD milking system. The reasons for unfavourable maternal heterosis effects on SMFS, FSCO and CFS have not been identified in this study.

At present, crossbred FxJ cows account almost half of the national herd in New Zealand. The reason of the rapid increase of numbers of crossbred cows in the national herd is due to their better production efficiency, fertility and improved health and survival (Montgomerie, 2005). Lopez-Villalobos et al. (2000) simulated the profitability of crossbreeding systems and found that a herd using a 2-breed rotational crossbreeding with F and J breeds showed superior farm profitability compared with straightbred herds. Clasen et al. (2020) used the phenotypic breed and heterosis effects to simulate the profitability of crossbreeding in two different production systems of organic and conventional herds in Sweden. Similarly, breed and heterosis effects reported for production and fertility traits in this study can also be used in simulation studies to design optimal crossbreeding strategies for OAD and TAD milking production systems.

4.6 Conclusions

The present study provided heritability and repeatability estimates for production and fertility traits of OAD the milking cow population in New Zealand. Individual and maternal heterosis and recombination losses were also estimated. Reported heritability and repeatability estimates in cows milked OAD were broadly similar to TAD milking cows. Importantly, most of the individual breed and heterosis effects for production and fertility traits were significant in both milking populations. The maternal heterosis effects for fertility traits of SMFS, FSCO, CFS, SR21 and SR42 were significant in cows milked TAD, but not in cows milked OAD. Recombination effects estimated in the present study were small and mix of favourable and unfavourable effects for production and fertility traits. The adoption of crossbreeding offers an opportunity for those farmers who are not already benefitting from heterosis to reap the benefits of improved milk production and fertility with minimal recombination loss.

4.7 References

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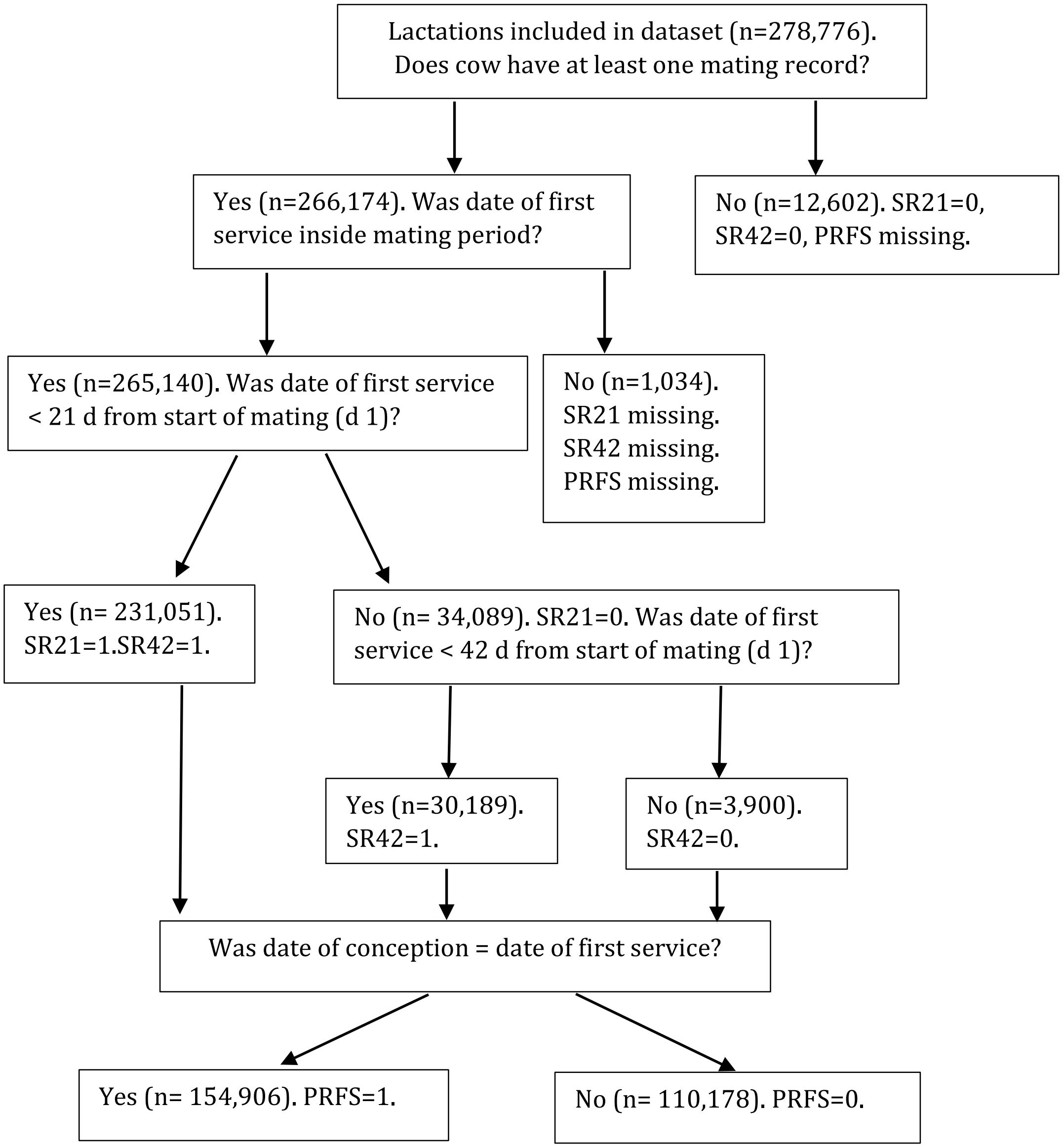
4.8 Annexure



Annexure 1. Flowchart detailing determination of 3-wk submission (SR21), 6-wk submission (SR42) and conception to the first service (PRFS).

Chapter 5

Heritabilities and genetic and phenotypic correlations for milk production and fertility traits of spring-calved once daily or twice daily milking cows in New Zealand

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5.1 Abstract

The objectives of this study were to estimate the genetic and phenotypic correlations and heritabilities for milk production and fertility traits in spring-calved once daily (OAD) milking cows for the whole season in New Zealand and compare those estimates with twice daily (TAD) milking cows. Data used in the study consisted of 69,252 first parity cows from the calving seasons 2015-2016 to 2017-2018 in 113 OAD and 531 TAD milking herds. Heritability estimates for production and fertility traits were obtained through single-trait animal models and estimates of genetic and phenotypic correlations were obtained through bivariate animal models. Heritability estimates of production traits varied from 0.26 to 0.61 in OAD and from 0.13 to 0.63 in TAD. Heritability estimates for fertility traits were low in both OAD and TAD milking cow populations and estimates were consistent (OAD: 0.01 to 0.10 and TAD: 0.01 to 0.08) across milking regimens. Estimates of phenotypic and genetic correlations among production traits were consistent across populations. In both populations phenotypic correlations between milk production and fertility traits were close to zero and most of the genetic correlations were antagonistic. In OAD milking cows, genetic correlations of milk and lactose yields with start of mating to conception, 6-wk in-calf, not-in-calf and 6-wk calving rate were close to zero. Interval from first service to conception was negative genetically correlated with milk and lactose yields in OAD milking cows. Protein percentage was positively genetically correlated with 3-wk and 6-wk submission, 3-wk in-calf, 6-wk in-calf, first service to conception, 3-wk calving and 6-wk calving in the TAD milking cow population, but these correlations were low in the OAD milking cow population. Further studies are needed to understand the relationship of PP and fertility traits in OAD milking system. The phenotypic correlations between fertility traits were similar in OAD and TAD milking populations. Genetic correlations between fertility traits were strong (≥0.70) in cows milked TAD but genetic correlations varied from weak to strong in cows milked OAD. Further research is required to evaluate the interaction between genotype by milking regimen for fertility traits in terms of sire selection in OAD milking cow population.

Key words: fertility, milk composition, once daily milking, genetic correlation

5.2 Introduction

Genetic and phenotypic correlations between milk production and fertility measures of dairy cows have been well studied in seasonal (Grosshans et al., 1997; Pryce et al., 1997; Evans et al., 2002; Haile-Mariam et al., 2003) and non-seasonal dairy production systems (Campos et al., 1994; Castillo-Juarez et al., 2000; Tiezzi et al., 2012). The main conclusion from the cited studies is that genetic correlations between milk production and fertility traits are antagonistic, indicating that selection for increased milk production results in genetically less fertile cows. Consequently, most dairy cattle breeding programs have incorporated fertility in their selection indices (Miglior et al., 2005). In New Zealand, fertility has been included in the Breeding Worth (BW) index since 2001 (Harris and Montgomerie, 2001).

The New Zealand dairy production system is pasture-based and has a seasonally concentrated calving pattern with fixed calendar dates for the start of mating and calving in a year. The proportion of cows that conceive in the first 6-wk of the mating season (6-wk in-calf rate; **PR42**) is one of the key reproductive indicators in seasonal calving herds. This indicator is an outcome of both 3-wk submission (**SR21**) and conception to first service (**PRFS**) (Xu and Burton, 2003). In New Zealand, national averages for SR21, PRFS and PR42 have improved from 78.5%, 50.1% and 63.4% in 2008-2009 to 81.3%, 52.7% and 67.7% in the 2020-2021 production seasons, respectively (LIC and DairyNZ, 2021). This improvement in reproductive performance of the national herd is likely to be caused by both managerial factors and increases in fertility breeding values (Pryce et al., 2014).

Since the late 1990s, some farmers have adopted once daily (**OAD**) milking for the whole lactation in New Zealand (Davis et al., 1999). For the production season 2015-2016, about 55% of herd-tested farms were milked twice daily (**TAD**) for the entire lactation whereas about 9% of herds were milked OAD for the entire lactation, and the remainder of farms practiced a mixture of TAD and OAD milking (Edwards 2018). Studies in New Zealand reported that cows milked OAD had improved energy balance in early lactation (Kay et al., 2012; Phyn et al., 2014) and cows milked OAD for the entire lactation had better reproductive performance than cows milked TAD (Jayawardana et al., 2022). Lembeye et al. (2016) reported genetic and phenotypic correlations between milk yields and milk composition traits in OAD milking cows in New Zealand. But so far, the genetic and phenotypic associations between production and fertility traits have not been calculated for the OAD milking cow population.

In New Zealand, Harris and Pryce (2004) reported significant phenotypic associations and low genetic correlations between milk protein percentage and both SR21 and the probability of having a calf born to artificial breeding (**AB**). Positive phenotypic and genetic associations between milk composition and fertility traits have been reported in seasonal and split-calving Australian herds (Fahey et al., 2017; Haile-Mariam and Pryce, 2017; Morton et al., 2018) and in spring-calving herds in Ireland (Buckley et al., 2003; Carty et al., 2020). Morton et al. (2018) reported that the association between milk protein concentration and fertility is influenced by both genetic and nongenetic components and cow reproductive performance can be maximized by improving genetic and non-genetic management strategies. To provide strategies for farmers to improve reproductive performance it is important to understand the genetic and phenotypic correlations between milk composition and fertility traits in dairy cows milked OAD or TAD.

Lembeye et al. (2021) reported the genetic correlations for same milk production traits expressed across the OAD and TAD milking frequency were strong but lower than unity. It is of interest to investigate the genetic correlations of milk production and fertility traits in OAD and TAD milking cow populations since common sires are used in both milking environments. The genetic correlations between milk production and fertility traits could be different in OAD compared with TAD milking populations, since the extent of negative energy balance in early lactation is reduced for cows milked OAD than TAD. Therefore, the objective of this study was i) to estimate the genetic and phenotypic correlations and heritabilities for milk production and fertility traits in cows milked OAD for the whole season in New Zealand, and ii) to compare the estimated genetic and phenotypic correlations for cows milked OAD with those for cows milked TAD.

5.3 Materials and methods

The data used in this study was collected by farmers as part of normal farming practice and retrospectively extracted and analysed. Therefore, animal ethics approval was not required for this study.

5.3.1 Initial Data set

Herd-test milk yields, calving, mating and pregnancy diagnosis dates, lactation yields, and pedigree information of first parity spring-calved dairy cows in three calving seasons from 2015-2016 to 2017-2018 in New Zealand were extracted from Livestock Improvement Corporation (**LIC**) database. Herd milking regimen classification and editing of milk production and fertility data was described in Jayawardana et al. (2023). Herds were included in this study if they had at least 50 cows in a herd, were herd tested at least four times per lactation in each calving season, had pregnancy diagnosis (**PD**) test results for at least 80% of cows that calved in the 12-month period and had early aged PD results for at least 80% of cows in a herd. The resulting data set consisted of 488,946 spring-calving first-lactation dairy cows in 2,421 herds.

5.3.2 Herd classification

Herd-test day milking regimen was used to classify the herds into OAD or TAD milking categories. If more than 90% of the tested cows in a herd at a herd-testing date were milked OAD, then the herd was classified as being OAD at that test date. Similarly, TAD milking herds were identified as those where more than 90% of the cows were milked TAD in the herd test date. If all the herd tests were classified as either OAD or TAD from 2015-2016 to 2017-2018 calving seasons, then the herd was identified as OAD or TAD throughout period, respectively. All other herds were excluded. Using the GPS Visualizer (Schneider 2012), peer TAD milking herds were selected within a radius of 20 km from a OAD milking herd. In some cases, there were no TAD milking herds within

20 km from an OAD milking herd. These OAD milking herds (n=8) were excluded from the data set. There were 114 OAD and 554 TAD herds comprising 17,134 of OAD milking and 86,233 of TAD milking cows.

5.3.3 Breed and heterosis information

Pedigree information with breed composition (expressed in sixteenths) was used to classify the cows into three breed groups: Holstein Friesian (**F**), Jersey (**J**) and crossbred of Holstein Friesian and Jersey (**F×J**). All cows in the data set had 100% known breed composition and no more than 12.5% of any breed other than the F or J. Herds that did not have F, J, and crossbred F×J cows were excluded (n=3 TAD herds). Coefficients of individual and maternal heterosis were calculated for each cow using the following equation (Dickerson, 1973):

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

Where calculation of individual heterosis coefficient for progeny, α_F^S and α_J^S are breed proportions of F and J in the sire, respectively and α_J^D and α_F^D are breed proportions of J and F in the dam, respectively. The calculation of maternal heterosis coefficient for dam α_F^S and α_J^S are breed proportions of F and J in the maternal grandsire and α_J^D and α_F^D are breed proportions of J and F in the maternal granddam, respectively.

5.3.4 Milk production traits

Lactation records of milk (**MY**), fat (**FY**), protein (**PY**), and lactose (**LY**) yields (kg) with days in milk ranging from 150 d to 305 d were included. Cows with MY greater than 12,500 kg or less than 800 kg, or FY, PY, and LY greater than 600 kg or less than 30 kg, were excluded (n=3,264). Percentages of fat (**FP**) and protein (**PP**) and lactose (**LP**) were calculated for each lactation record as the ratio between FY, PY, or LY and MY multiplied by 100.

5.3.5 Fertility traits

Calving dates between 1st June and 31st December in each calving season were retained. Cows with age at first calving that was greater than 900 d were excluded. The mating start and end dates for each herd were obtained from the LIC database. The mating start date of a herd was defined as the first of two consecutive days, both with at least one mating recorded, where at least 3 of the next 6 days also have mating records. The mating end date of the herd was defined as either i) the last recorded mating date on or before the 21st wk of the mating period or, ii) the last date with two conceptions followed by at least 30 d with no conceptions on or before the 21st wk of the mating period or, iii) 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 two or more other days in the 6 preceding days. Herds with the start of the mating before the 15th of September in each spring-calving season were excluded (n=1 in OAD and n=21 in TAD). Mating date records outside the herd's breeding season were excluded from the calculation of fertility traits due to uncertainty of those records (n=264). Cows that were missing both a PD and all herd tests after the end of the mating period were considered to have left the herd prior to the end of mating and were discarded from the data set (n=2,164).

Conception dates were determined from the results of transrectal ultrasound or rectal palpation PD. Pregnancy testing results where the foetal age was estimated and where cows were tested on or between 35-122 d from conception were used. Conception dates were calculated as the date of pregnancy diagnosis minus the estimated foetal age for cows with a pregnancy status of 'pregnant'(n=47,742). Conception dates were also calculated for cows without recorded PD results but which calved in the subsequent season as the date of subsequent calving minus 282 d (n=1,514). The detailed description for the calculation of conception date, 3-wk in-calf (**PR21**), PR42 and not in-calf (**NIC**) for cows in all parities was described in Figure 4.3 in Jayawardana et al. (2023).

Table 5.1 defines the fertility traits and the criteria for trait calculations in the present study. Cows with no recorded AB inseminations (n=2,653) were allocated the end date of the AB period in the herd as a penalised first service date. The end of the AB period was identified in each herd as the date of the last AB insemination that was not followed by another AB insemination within 7 days. Penalised conception dates for cows that did not conceive (n=8,093) were allocated as the mating end date in each herd plus 21 d (Johnston and Bunter, 1996; Grosshans et al., 1997). The planned start of calving (**PSC**) date for subsequent calving year was derived for each herd by adding 282 d to the herd's mating start date in each calving year.

The final data set used for genetic parameters estimation comprised 10,709 first parity cows from 113 OAD herds and 58,543 first parity cows from 531 TAD herds. The distribution of cows by breed in OAD and TAD herds was: 1,362 (12.7%) F, 2,791 (26.1%) J and 6,556 (61.2%) F×J cows in OAD and 25,297 (43.2%) F, 4,351 (7.4%) J and 28,895 (49.4%) F×J cows in TAD.

Table 5.1. Description of fertility traits and criteria for calculation

Abbreviation	Description of traits and criteria for calculation
Production traits	
MY	305-day milk production (kg)
FY	305-day fat production (kg)
PY	305-day protein production (kg)
LY	305-day lactose production (kg)
FP	Fat percentage, kg fat per kg milk (%)
PP	Protein percentage, kg protein per kg milk (%)
LP	Lactose percentage, kg lactose per kg milk (%)
Fertility traits	
SMFS[1]	Interval from the start of mating to the first service (d)
SMCO[2]	Interval from the start of mating to conception (d)
FSCO[2]	Interval from the first service to conception (d)
CFS[1]	Interval from calving to first service (d)
CI[3]	Interval between two consecutive calvings (d)
SR21	Presentation (1) or non-presentation (0) for mating in the first 21 d from the start of the mating date
SR42	Presentation (1) or non-presentation (0) for mating in the first 42 d from the start of the mating date
PR21	Conceived (1) or not conceived (0) in the first 21 d from start of mating date
PR42	Conceived (1) or not conceived (0) in the first 42 d from start of mating date
PRFS	Conceived (1) or not conceived (0) to first service
NIC	Conceived (0) or not conceived (1) by end of the mating period
CR21[4]	Calved within the first 21 d of the planned start of calving date herd in the subsequent season (1) or calved after the first 21 d from the planned start of calving in the subsequent season (0)
CR42[4]	Calved within the first 42 d of the planned start of calving date herd in the subsequent season (1) or calved after the first 42 d from the planned start of calving in the subsequent season (0)

[1] Intervals for cows with no recorded artificial breeding (AB) were ended at the end of the AB period of herd in each calving season.

[2] Intervals for cows that did not conceive were ended at the herd's end of the mating date plus 21 days.

[3] Intervals for cows with positive pregnancy diagnosis results and without subsequent calving dates were calculated by estimating a calving date using conception date plus 282 days.

[4] Cows that did not calve in the subsequent season were treated as missing variables.

5.3.6 Estimation of genetic parameters

Statistical data analysis was carried out using the ASReml version 4.1 software package (Gilmour et al., 2015). Variance components and heritability were estimated using a single-trait animal model and bivariate animal models were used separately to estimate the (co)variance components, genetic and phenotypic correlations. Contemporary groups were defined for cows in the same herd and calving year as herd-year. The single-trait animal model was represented as follows:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{e}$$

Where $\boldsymbol{y}$ is the vector of observations for each production and fertility traits in Table 5.1; $\boldsymbol{b}$ is the vector of fixed effects, $\boldsymbol{a}$ is the vector of additive genetic effects, $\boldsymbol{e}$ is the vector of random residual effects. **X** and **Z** are incidence matrices relating to the records of fixed and animal additive genetic effects, respectively.

The fixed effects were contemporary group of herd-year; the regression coefficients related to the linear effect of the proportion of F in the cow and dam, respectively (proportion of J for cow and dam was not included in the model due to the linear dependencies); the regression coefficients related to the linear effect individual and maternal heterosis coefficients, respectively; the regression coefficients related to the linear and quadratic effects of deviation of calving date from median calving date of the herd within the season and the regression coefficients related to linear and quadratic effects of deviation of age at first calving from median calving in months. Logit scale was used to estimate the variance components related to binary fertility traits in Table 5.1 with the random residual variance of $\pi^2/3 = 3.2899$.

The expected values (E) of the variables were assumed as E(y)=Xb, E(a)=0 and E(e)=0. The residuals were assumed to be independently distributed and var($\mathbf{y}$)=$\mathbf{ZAZ'A}\sigma_a^2 + \mathbf{R}$, var(a)=$\mathbf{A}\sigma_a^2$ and var(e)=$\mathbf{I}\sigma_e^2$= $\mathbf{R}$; where σ_a^2 was the animal additive genetic variance, σ_e^2 was the random residual variance, **A** was the numerator relationship matrix between all the cows and **I** was the identity matrix which corresponds to the number of cows with records.

Heritability (h^2) for each trait was calculated using the following ratio (Falconer and Mackay, 1996):

$$h^2 = \sigma_a^2/\sigma_p^2$$

Where: σ_p^2 was the phenotypic variance, similar to $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$

In matrix notation, the bivariate model was represented as follows:

$$\begin{bmatrix} \mathbf{y_1} \\ \mathbf{y_2} \end{bmatrix} = \begin{bmatrix} \mathbf{X_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{X_2} \end{bmatrix} \begin{bmatrix} \mathbf{b_1} \\ \mathbf{b_2} \end{bmatrix} + \begin{bmatrix} \mathbf{Z_1} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z_2} \end{bmatrix} \begin{bmatrix} \mathbf{a_1} \\ \mathbf{a_2} \end{bmatrix} + \begin{bmatrix} \mathbf{e_1} \\ \mathbf{e_2} \end{bmatrix}$$

Where: $\mathbf{y_1}$ and $\mathbf{y_2}$ are the vectors of phenotypic measures for two traits;

$\mathbf{X_1}$, $\mathbf{X_2}$, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ were design matrices relating the fixed and animal additive genetic effects related to the $\mathbf{y_1}$and $\mathbf{y_2}$ phenotypes, respectively;

$\mathbf{b_1}$ and $\mathbf{b_2}$ were the vectors of fixed effects of herd-year, the regression coefficients related to the linear effect of the proportion of F in the cow and dam, respectively, the regression coefficients related to the linear effect of individual and maternal heterosis coefficients, respectively, the regression coefficients related to the linear and quadratic effects of deviation of calving date from median calving date of the herd in the given calving season and the regression coefficient related to the linear effect of deviation of age at first calving from median calving in months;

$\mathbf{a_1}$ and $\mathbf{a_2}$ were the vectors of random effects of animal for each trait; and $\mathbf{e_1}$ and $\mathbf{e_2}$ were vectors of residual errors.

The expected values of the variables were assumed $E(y_1) = X_1 b_1$; $E(y_2) = X_2 b_2$; $E(a)=0$ and $E(e)=0$. The residuals were assumed to be normally distributed with zero mean and following co(variance) structure:

$$\text{var}\begin{bmatrix} a_1 \\ a_2 \\ e_1 \\ e_2 \end{bmatrix} = \begin{bmatrix} A\sigma_{a1}^2 & A\sigma_{a12} & 0 & 0 \\ A\sigma_{a12} & A\sigma_{a2}^2 & 0 & 0 \\ 0 & 0 & I\sigma_{e1}^2 & I\sigma_{e12} \\ 0 & 0 & I\sigma_{e12} & I\sigma_{e2}^2 \end{bmatrix}$$

Where: σ_{a1}^2 was the additive genetic variance of the trait 1; σ_{a2}^2 was the additive genetic variance of trait 2 and σ_{a12} was the additive genetic covariance between both traits. I was an identity matrix of size 10,709 and 58,543 for OAD and TAD populations; σ_{e1}^2 was the residual variance for trait 1; σ_{e2}^2 was the residual variance of trait 2 and σ_{e12} was the residual covariance between both traits.

Genetic correlations (r_g) were estimated as (Falconer and Mackay, 1996):

$$r_g = \frac{\sigma_{a12}}{\sigma_{a1} \times \sigma_{a2}}$$

Where: σ_{a1} and σ_{a2} were genetic additive standard deviation for trait 1 and trait 2, respectively.

Phenotypic correlations (r_P) were estimated as (Falconer and Mackay, 1996):

$$r_P = \frac{\sigma_{P12}}{\sigma_{P1} \times \sigma_{P2}}$$

Where: σ_{P12} was phenotypic covariance between phenotypic trait 1 and trait 2; σ_{P1} and σ_{P2}were phenotypic standard deviation for trait 1 and trait 2, respectively.

5.4 Results

5.4.1 Reproductive performance

Descriptive statistics of production and fertility traits, by milking frequency are shown in Table 5.2. First parity cows milked TAD had greater ($P < 0.001$) yields of milk, fat, protein and lactose compared with cows milked OAD, but with lower ($P < 0.001$) percentages of fat and protein and higher ($P < 0.001$) percentage of lactose. Cows milked OAD had improved reproductive performance compared with cows milked TAD with shorter ($P < 0.001$) intervals from the start of mating to first service (**SMFS**: 1.4 d), start of mating to conception (**SMCO**: 6.6 d), first service to conception (**FSCO**: 5.4 d) and consecutive calving interval (**CI**: 3.5 d). Higher proportions ($P < 0.001$) of cows that were milked OAD were mated (6%) and conceived (11%) in the first 3-wk of the mating period compared with cows milked TAD. Also, PRFS was higher ($P < 0.001$) in OAD than TAD. Consequently, reproductive performance as measured by, PR42 (higher, $P < 0.001$) and NIC (lower, $P < 0.001$) was superior in cows milked OAD. The proportion of cows calved by 3-wk (**CR21**) and calved by 6-wk (**CR42**) were also greater ($P < 0.001$) in cows milked OAD compared with cows milked TAD.

Table 5.2. Descriptive statistics of milk production and fertility traits by milking regimen in first lactating dairy cows in New Zealand

Trait	Once daily milking			Twice daily milking		
	Mean	SD	CV	Mean	SD	CV
Production[1]						
MY	2456^{b}	631	26	4158^{a}	1057	25
FY	133^{b}	33	25	197^{a}	48	24
PY	102^{b}	26	25	160^{a}	40	25
LY	123^{b}	32	26	212^{a}	55	26
FP	5.5^{a}	0.7	12.4	4.8^{b}	0.7	14.5
PP	4.2^{a}	0.3	6.9	3.9^{b}	0.3	7.5
LP	5.0^{b}	0.2	3.6	5.1^{a}	0.2	3.5
Fertility[2]						
SMFS	12.2^{b}	10.8	88.4	13.6^{a}	13.7	100.6
SMCO	25.9^{b}	22.5	87.0	32.5^{a}	26.7	82.0
FSCO	13.9^{b}	20.9	149.8	19.4^{a}	25.1	129.5
CFS	85.8^{a}	20.1	23.5	86.4^{a}	20.6	23.8
CI	375.0^{b}	23.4	6.2	378.5^{a}	25.3	6.7
SR21	0.89^{a}	0.31	35.3	0.83^{b}	0.38	45.7
SR42	0.95^{a}	0.22	22.7	0.93^{a}	0.25	26.4
PR21	0.59^{a}	0.49	84.0	0.48^{b}	0.50	104.2
PR42	0.79^{a}	0.41	51.6	0.69^{b}	0.46	66.9
PRFS	0.65^{a}	0.48	72.7	0.57^{b}	0.49	86.0
NIC	0.09^{b}	0.28	323.5	0.13^{a}	0.34	259.8
CR21	0.67^{a}	0.47	69.6	0.60^{b}	0.49	82.3
CR42	0.89^{a}	0.32	35.9	0.82^{b}	0.38	46.5

[a,b] Means with different superscripts, with in the same row are significantly different ($P < 0.001$). Models were fitted for each trait with fixed effects of milking regimen, the proportions of Holstein Friesian in cow and dam, respectively, the coefficients of individual and maternal heterosis, respectively. The deviation of calving date from median calving date of the herd within the season (linear and quadratic effects) and the deviation of age at first calving from median calving in months (linear and quadratic effects) were fitted as covariates in the models. Herd-year was fitted as a random effect.

[1] MY = milk yield (kg); FY = fat yield (kg); PY= protein yield (kg); LY = lactose yield (kg); FP = fat percentage; PP = protein percentage; LP = lactose percentage.

[2] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); 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.

5.4.2 Variance components and heritability

Estimates of variance components and heritabilities for production and fertility traits of first parity dairy cows milked OAD and TAD populations are presented in Table 5.3.

Table 5.3. Estimates of variances and heritabilities with standard errors for production and fertility traits of the first parity cows milked once daily (OAD) and twice daily (TAD) in New Zealand

Trait	OAD milking					TAD milking				
	Variance			Heritability	SE	Variance			Heritability	SE
	Residual	Genetic	Total			Residual	Genetic	Total		
Production[1]										
MY	148,671	77,622	226,293	0.34	0.04	344,268	103,557	447,825	0.23	0.02
FY	486.6	166.9	653.5	0.26	0.04	805.5	182.2	987.6	0.18	0.02
PY	273.7	102.2	375.9	0.27	0.04	499.8	75.7	575.5	0.13	0.01
LY	399.7	216.7	616.4	0.35	0.04	938.0	273.3	1211.2	0.23	0.02
FP	0.14	0.17	0.31	0.56	0.05	0.11	0.18	0.28	0.63	0.02
PP	0.02	0.03	0.05	0.61	0.05	0.02	0.03	0.05	0.59	0.03
LP	0.02	0.01	0.03	0.29	0.04	0.02	0.01	0.025	0.30	0.02
Fertility[2]										
SMFS	104.0	2.4	106.4	0.02	0.01	145.9	4.20	150.1	0.03	0.01
SMCO	676.0	21.2	697.2	0.03	0.02	917.2	37.3	954.5	0.04	0.01
FSCO	412.3	7.9	420.2	0.02	0.01	576.7	9.5	586.2	0.02	<0.01
CFS	104.0	2.4	106.4	0.02	0.01	145.9	4.2	150.1	0.03	<0.01
CI	309.5	8.9	318.4	0.03	0.01	394.7	8.4	403.1	0.02	<0.01
SR21	3.29	0.35	3.64	0.10	0.02	3.29	0.23	3.52	0.07	0.01
SR42	3.29	0.28	3.57	0.08	0.04	3.29	0.29	3.58	0.08	0.02
PR21	3.29	0.12	3.41	0.04	0.01	3.29	0.10	3.39	0.03	0.01
PR42	3.29	0.13	3.42	0.04	0.02	3.29	0.13	3.42	0.04	0.01
PRFS	3.29	0.05	3.34	0.01	0.01	3.29	0.05	3.34	0.01	<0.01
NIC	3.29	0.23	3.52	0.07	0.03	3.29	0.16	3.45	0.05	0.01
CR21	3.29	0.11	3.40	0.03	0.02	3.29	0.09	3.38	0.03	0.01
CR42	3.29	0.04	3.33	0.01	0.03	3.29	0.12	3.41	0.03	0.01

[1] MY = milk yield (kg); FY = fat yield (kg); PY= protein yield (kg); LY = lactose yield (kg); FP = fat percentage; PP = protein percentage; LP = lactose percentage.

[2] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); 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.

Heritability estimates for yields (MY, FY, PY and LY) in the OAD population were greater than in the TAD population. Heritability estimates of milk composition traits were similar in cows milked either OAD or TAD. Heritabilities of FP and PP were more than twice as large as estimates for the respective yield traits (FY and PY) in both milking populations. The estimates of heritability for LP were similar in both milking populations and were much lower than the heritability estimates for FP and PP.

Heritability estimates of fertility traits were lower than the heritability estimates for production traits in both milking populations. Heritability estimates for fertility traits ranged from 0.01 to 0.10 in OAD and from 0.01 to 0.08 in the TAD milking population. The highest heritability estimate was for SR21 and 6-wk submission (**SR42**) (0.1 and 0.08, respectively) in OAD and TAD milking populations, respectively. The heritability estimate for PRFS was lowest (0.01) in both milking populations. Overall, heritability estimates for fertility traits were similar between OAD and TAD milking populations.

Genetic and phenotypic correlations between yield, composition, and fertility traits for OAD and TAD milking cow populations are shown in Table 5.4 and Table 5.5, respectively.

Table 5.4. Genetic (below the diagonal with standard errors in parentheses) and phenotypic (above the diagonal) correlations between milk production and fertility traits in once daily (OAD) milking cows

Trait[1]	MY	FY	PY	LY	FP	PP	LP	SMFS	SMCO	FSCO
MY		0.85	0.95	0.99	-0.26	-0.19	0.20	0.03	-0.02	-0.03
FY	0.71 (0.04)		0.90	0.84	0.27	0.11	0.17	0.01	-0.04	-0.04
PY	0.92 (0.01)	0.84 (0.03)		0.95	-0.10	0.10	0.20	0.01	-0.04	-0.04
LY	0.99 (0.01)	0.71 (0.04)	0.92 (0.01)		-0.26	-0.19	0.34	0.03	-0.02	-0.03
FP	-0.50 (0.06)	0.25 (0.08)	-0.23 (0.08)	-0.49 (0.06)		0.57	-0.06	-0.04	-0.03	-0.01
PP	-0.40 (0.07)	0.15 (0.08)	-0.01 (0.08)	-0.39 (0.07)	0.76 (0.03)		-0.02	-0.07	-0.08	-0.05
LP	0.26 (0.09)	0.25 (0.09)	0.31 (0.09)	0.39 (0.08)	-0.06 (0.08)	0.01 (0.08)		-0.02	-0.01	-0.01
SMFS	0.42 (0.18)	0.36 (0.10)	0.43 (0.19)	0.38 (0.18)	-0.02 (0.18)	-0.12 (0.18)	-0.11 (0.19)		0.31	-0.09
SMCO	0.08 (0.18)	0.19 (0.18)	0.14 (0.18)	0.07 (0.18)	0.14 (0.16)	0.12 (0.16)	-0.16 (0.18)	0.42 (0.26)		0.93
FSCO	-0.19 (0.22)	0.01 (0.22)	-0.09 (0.22)	-0.17 (0.21)	0.23 (0.21)	0.29 (0.21)	-0.12 (0.22)	-0.02 (0.35)	0.96 (0.04)	
CFS	0.42 (0.18)	0.36 (0.10)	0.43 (0.19)	0.38 (0.18)	-0.02 (0.18)	-0.12 (0.18)	-0.11 (0.19)	-	0.42 (0.26)	-0.02 (0.35)
CI	0.21 (0.19)	0.23 (0.19)	0.20 (0.19)	0.23 (0.19)	-0.001(0.18)	-0.10 (0.17)	0.08 (0.19)	0.88 (0.12)	0.99 (0.01)	0.96 (0.04)
SR21	-0.53 (0.14)	-0.39 (0.15)	-0.51 (0.14)	-0.52 (0.14)	0.16 (0.14)	0.26 (0.15)	-0.04 (0.16)	-0.99 (0.01)	-0.67 (0.18)	-0.34 (0.27)
SR42	-0.39 (0.23)	-0.42 (0.24)	-0.41 (0.24)	-0.36 (0.23)	-0.03 (0.23)	0.06 (0.23)	-0.02 (0.24)	-0.98 (0.02)	-0.52 (0.32)	0.11 (0.43)
PR21	-0.25 (0.18)	-0.30 (0.18)	-0.27 (0.18)	-0.30 (0.17)	-0.04 (0.17)	0.05 (0.16)	-0.19 (0.17)	-0.70 (0.25)	-0.93 (0.07)	-0.80 (0.11)
PR42	-0.07 (0.20)	-0.22 (0.21)	-0.13 (0.21)	-0.08 (0.20)	-0.18 (0.19)	-0.16 (0.19)	0.05 (0.20)	-0.14 (0.32)	-0.99 (0.01)	-0.92 (0.04)
PRFS	-0.16 (0.36)	-0.17 (0.36)	-0.21 (0.37)	-0.20 (0.36)	0.06 (0.32)	-0.08 (0.33)	-0.03 (0.33)	-0.02 (0.47)	-0.84 (0.09)	-0.94 (0.06)
NIC	0.06 (0.20)	0.21 (0.20)	0.13 (0.20)	0.03 (0.19)	0.16 (0.18)	0.15 (0.18)	-0.30 (0.20)	0.08 (0.33)	0.89 (0.09)	0.51 (0.47)
CR21	-0.55 (0.24)	-0.52 (0.24)	-0.54 (0.24)	-0.63 (0.22)	0.11 (0.23)	0.17 (0.23)	-0.60 (0.22)	-0.73 (0.30)	-0.99 (0.01)	-0.86 (0.12)
CR42	-0.06 (0.16)	-0.02 (0.16)	0.01 (0.19)	-0.05 (0.16)	-0.01(0.16)	0.02 (0.19)	-0.28 (0.19)	-0.71 (0.30)	-0.98 (0.01)	-0.91 (0.12)

Table 5.4. Genetic (below the diagonal with standard errors in parentheses) and phenotypic (above the diagonal) correlations between milk production and fertility traits in once daily (OAD) milking cows (continued)

Trait[1]	CFS	CI	SR21	SR42	PR21	PR42	PRFS	NIC	CR21	CR42
MY	0.03	0.03	-0.05	-0.02	-0.02	0.01	0.01	-0.02	-0.06	-0.04
FY	0.01	0.01	-0.02	-0.002	-0.001	0.02	0.01	-0.03	-0.04	-0.02
PY	0.01	0.01	-0.03	-0.01	0.0001	0.03	0.02	-0.04	-0.05	-0.03
LY	0.03	0.02	-0.04	-0.01	-0.01	0.01	0.01	-0.02	-0.06	-0.03
FP	-0.04	-0.03	0.05	0.03	0.03	0.01	0.02	-0.02	0.02	0.03
PP	-0.07	-0.06	0.06	0.04	0.06	0.06	0.05	-0.07	0.04	0.03
LP	-0.02	-0.02	0.02	0.01	0.02	0.01	0.01	0.003	0.005	0.01
SMFS	-	0.37	-0.73	-0.61	-0.27	-0.23	0.12	0.14	-0.28	-0.16
SMCO	0.31	0.95	-0.26	-0.20	-0.77	-0.86	-0.57	0.73	-0.86	-0.81
FSCO	-0.09	0.88	0.04	0.07	-0.77	-0.85	-0.64	0.63	-0.80	-0.78
CFS		0.37	-0.73	-0.61	-0.27	-0.22	0.12	0.14	-0.27	-0.16
CI	0.88 (0.12)		-0.28	-0.19	-0.77	-0.76	-0.46	0.28	-0.81	-0.72
SR21	-0.99 (0.01)	-0.80 0.17)		0.62	0.37	0.23	-0.06	-0.10	0.30	0.19
SR42	-0.98 (0.02)	-0.74 (0.13)	0.71 (0.23)		0.18	0.21	-0.02	-0.10	0.18	0.16
PR21	-0.70 (0.25)	-0.97 (0.05)	0.79 (0.17)	0.75 (0.30)		0.60	0.62	-0.34	0.82	0.47
PR42	-0.14 (0.32)	-0.94 (0.06)	0.53 (0.23)	0.23 (0.38)	0.96 (0.04)		0.44	-0.59	0.58	0.82
PRFS	-0.02 (0.47)	-0.86 (0.02)	0.17 (0.41)	-0.17 (0.63)	0.78 (0.22)	0.99 (0.01)		-0.42	0.51	-
NIC	0.08 (0.33)	0.79 (0.17)	-0.38 (0.23)	-0.33 (0.36)	-0.62 (0.21)	-0.99 (0.01)	-0.47 (0.50)		-0.09	-
CR21	-0.73 (0.30)	-0.99 (0.01)	0.51 (0.29)	0.58 (0.43)	0.85 (0.09)	0.94 (0.04)	-0.46 (0.16)	-0.32 (0.39)		-
CR42	-0.71 (0.30)	-	-0.63 (0.45)	-0.50 (0.45)	0.30 (0.71)	0.85 (0.09)	-	-	-	

Standard errors for all phenotypic correlation estimates were ≤ 0.01. All estimates were significantly different from zero and non-significant or non-converged estimates were not represented.

[1] MY = milk yield (kg); FY = fat yield (kg); PY= protein yield (kg); LY = lactose yield (kg); FP = fat percentage; PP = protein percentage; LP = lactose percentage; SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); 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

Table 5.5. Genetic (below the diagonal with standard errors in parentheses) and phenotypic (above the diagonal) correlations between milk production and fertility traits in twice daily (TAD) milking cows

Trait[1]	MY	FY	PY	LY	FP	PP	LP	SMFS	SMCO	FSCO
MY		0.74	0.93	0.98	-0.34	-0.34	0.01	0.002	0.01	0.02
FY	0.29 (0.05)		0.82	0.73	0.35	0.07	0.01	-0.01	-0.02	-0.004
PY	0.81 (0.01)	0.51 (0.04)		0.91	-0.13	0.02	0.01	-0.01	-0.02	-0.01
LY	0.98 (0.01)	0.29 (0.05)	0.79 (0.02)		-0.33	-0.33	0.19	-0.004	0.004	0.01
FP	-0.65 (0.03)	0.53 (0.03)	-0.33 (0.05)	-0.63 (0.03)		0.60	-0.002	-0.03	-0.05	-0.03
PP	-0.70 (0.02)	0.15 (0.04)	-0.16 (0.05)	-0.69 (0.03)	0.72 (0.01)		0.001	-0.05	-0.09	-0.07
LP	-0.11 (0.05)	-0.03 (0.05)	-0.13 (0.06)	0.13 (0.05)	0.05 (0.04)	-0.01 (0.04)		-0.03	-0.04	-0.03
SMFS	0.26 (0.09)	0.18 (0.09)	0.22 (0.09)	0.23 (0.09)	-0.17 (0.08)	-0.20 (0.08)	-0.17 (0.09)		0.28	-0.15
SMCO	0.24 (0.08)	0.13 (0.09)	0.17 (0.09)	0.20 (0.08)	-0.10 (0.07)	-0.28 (0.07)	-0.12 (0.08)	0.89 (0.04)		0.92
FSCO	0.21 (0.11)	0.12 (0.10)	0.11 (0.10)	0.16 (0.10)	-0.06 (0.10)	-0.31 (0.09)	-0.14 (0.11)	0.71 (0.11)	0.98 (0.01)	
CFS	0.26 (0.09)	0.18 (0.09)	0.22 (0.09)	0.23 (0.09)	-0.17 (0.08)	-0.20 (0.08)	-0.17 (0.09)	-	0.89 (0.04)	0.71 (0.11)
CI	0.27 (0.10)	0.18 (0.10)	0.29 (0.10)	0.25 (0.10)	-0.10 (0.09)	-0.19 (0.09)	-0.11 (0.09)	0.83 (0.07)	0.98 (0.01)	0.95 (0.01)
SR21	-0.21 (0.08)	-0.18 (0.08)	-0.16 (0.09)	-0.19 (0.08)	0.10 (0.07)	0.22 (0.07)	0.17 (0.08)	-0.99 (0.01)	-0.87 (0.01)	-0.70 (0.10)
SR42	-0.17 (0.09)	-0.19 (0.10)	-0.14 (0.10)	-0.14 (0.10)	0.09 (0.09)	0.16 (0.09)	0.25 (0.09)	-0.98 (0.02)	-0.92 (0.04)	-0.75 (0.12)
PR21	-0.28 (0.09)	-0.18 (0.09)	-0.25 (0.09)	-0.24 (0.09)	0.11 (0.08)	0.24 (0.08)	0.16 (0.09)	-0.93 (0.04)	-0.97 (0.01)	-0.91 (0.03)
PR42	-0.28 (0.08)	-0.21 (0.09)	-0.23 (0.09)	-0.23 (0.09)	0.06 (0.08)	0.22 (0.08)	0.13 (0.09)	-0.89 (0.05)	-0.97 (0.01)	-0.98 (0.01)
PRFS	-0.35 (0.12)	-0.20 (0.13)	-0.30 (0.13)	-0.30 (0.13)	0.14 (0.12)	0.30 (0.11)	0.11 (0.13)	-0.77 (0.15)	-0.88 (0.05)	-0.85 (0.07)
NIC	0.25 (0.10)	0.08 (0.10)	0.12 (0.11)	0.20 (0.10)	-0.16 (0.09)	-0.35 (0.08)	-0.23 (0.10)	0.84 (0.06)	0.97 (0.02)	0.97 (0.03)
CR21	-0.26 (0.12)	-0.10 (0.13)	-0.27 (0.13)	-0.22 (0.12)	0.15 (0.11)	0.22 (0.11)	0.11 (0.12)	-0.77 (0.09)	-0.96 (0.02)	-0.91 (0.04)
CR42	-0.27 (0.13)	-0.23 (0.13)	-0.30 (0.14)	-0.24 (0.13)	0.12 (0.11)	0.20 (0.12)	0.10 (0.12)	-0.88 (0.10)	-0.99 (0.01)	-0.98 (0.02)

Table 5.5. Genetic (below the diagonal with standard errors in parentheses) and phenotypic (above the diagonal) correlations between milk production and fertility traits in twice daily (TAD) milking cows (continued)

Trait[1]	CFS	CI	SR21	SR42	PR21	PR42	PRFS	NIC	CR21	CR42
MY	0.002	0.05	-0.01	0.01	-0.03	-0.02	-0.03	0.001	-0.06	-0.05
FY	-0.02	0.03	0.01	0.02	-0.01	0.004	-0.01	-0.03	-0.04	-0.04
PY	-0.01	0.04	0.01	0.02	-0.01	0.01	-0.01	-0.03	-0.05	-0.04
LY	-0.004	0.04	-0.001	0.01	-0.03	-0.01	-0.02	-0.01	-0.05	-0.04
FP	-0.03	-0.03	0.04	0.02	0.04	0.04	0.02	-0.04	0.03	0.02
PP	-0.05	-0.05	0.06	0.04	0.07	0.08	0.04	-0.08	0.05	0.04
LP	-0.03	-0.03	0.04	0.03	0.04	0.04	0.01	-0.03	0.03	0.03
SMFS	-	0.34	-0.78	-0.68	-0.28	-0.23	0.12	0.14	-0.29	-0.21
SMCO	0.28	0.96	-0.26	-0.21	-0.75	-0.86	-0.55	0.74	-0.86	-0.85
FSCO	-0.15	0.89	0.07	0.10	-0.71	-0.84	-0.62	0.64	-0.77	-0.80
CFS		0.34	-0.78	-0.67	-0.28	-0.23	-0.06	0.14	-0.29	-0.20
CI	0.83 (0.07)		-0.31	-0.22	-0.76	-0.80	-0.45	0.20	-0.82	-0.78
SR21	-0.97 (0.03)	-0.80 (0.06)		0.54	0.38	0.25	0.06	-0.13	0.34	0.20
SR42	-0.98 (0.02)	-0.82 (0.08)	0.98 (0.02)		0.17	0.24	0.20	-0.13	0.17	0.23
PR21	-0.93 (0.04)	-0.93 (0.25)	0.89 (0.04)	0.91 (0.05)		0.62	0.61	-0.35	0.82	0.50
PR42	-0.89 (0.05)	-0.98 (0.01)	0.87 (0.05)	0.88 (0.05)	0.97 (0.02)		0.46	-0.56	0.59	0.85
PRFS	-0.77 (0.11)	-0.70 (0.10)	0.79 (0.10)	0.81 (0.11)	0.81 (0.06)	0.85 (0.06)		-0.42	0.52	0.29
NIC	0.84 (0.06)	0.85 (0.06)	-0.82 (0.06)	-0.85 (0.07)	-0.90 (0.04)	-0.94 (0.03)	-0.99 (0.01)		-0.13	-0.23
CR21	-0.79 (0.09)	-0.99 (0.01)	0.79 (0.09)	0.84 (0.09)	0.92 (0.03)	0.91 (0.04)	0.70 (0.13)	-0.74 (0.10)		0.54
CR42	-0.88 (0.10)	-0.99 (0.01)	0.86 (0.09)	0.93 (0.09)	0.97 (0.03)	0.99 (0.01)	0.73 (0.10)	-0.88 (0.08)	0.95 (0.05)	

Standard errors for all phenotypic correlation estimates were ≤ 0.01. All estimates were significantly different from zero.

[1] MY = milk yield (kg); FY = fat yield (kg); PY= protein yield (kg); LY = lactose yield (kg); FP = fat percentage; PP = protein percentage; LP = lactose percentage; SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); 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.

5.4.3 Correlations between the production traits

Milk yield was strongly positively genetically correlated with both PY and LY (≥ 0.81) in both milking populations. Genetic correlations of FY with MY, PY and LY were low to moderate in the TAD milking population (+0.29 to +0.51), but were strong (≥0.71) in the OAD milking population. Genetic and phenotypic correlations between MY and FP or PP were moderately negative in both milking populations. The genetic and phenotypic correlations between MY and LP were negative (-0.11) and close to zero (0.01) in the TAD milking population, but these correlations were moderately positive in the OAD milking population. The genetic correlation between FY and FP was moderately positive, but this estimate was stronger in the TAD milking population than in the OAD milking population. The genetic correlation between LY and LP was weak to moderately positive and was stronger in the OAD milking population than the TAD milking population. Estimated of genetic and phenotypic correlations between MY and LY were close to one in both milking populations.

In both milking populations, genetic and phenotypic correlations of MY, PY, LY with FP and PP had the opposite sign (negative) compared with the correlations of FY with FP and PP (positive). The genetic and phenotypic correlations between FP and PP were similar and moderate to high in both milking populations. However, the estimates of LP with FP and PP were closer to zero in both milking populations.

5.4.4 Correlations between production and fertility traits

In both milking populations, all the phenotypic correlations between milk production and fertility traits were weak and close to zero (OAD: -0.08 to 0.06; TAD: -0.09 to 0.08). Most of the genetic correlations between milk production and fertility traits were weak to moderately negative. The genetic and phenotypic correlations of SMFS and calving to first service (**CFS**) with other production and fertility traits were similar in across milking systems.

The genetic correlations between yield and interval fertility traits were low and positive in TAD (from +0.11 to +0.29). Genetic correlations of all yield traits (MY, FY, PY and LY) with SMFS and CFS were positive and relatively greater in cows milked OAD than in cows milked TAD. However, genetic correlations between MY, LY and SMCO were lower (nearly zero) in cows milked OAD compared with TAD. Estimated genetic correlations of MY, PY, LY with FSCO were negative for cows milked OAD, but these genetic correlations were positive in the TAD milking population. In both milking populations, genetic correlations between all yields and binary fertility traits were negative, except for NIC. The estimated genetic correlations of yield traits with SR21, SR42, and CR21 tended to be greater in cows milked OAD compared with cows milked TAD. However, genetic correlation estimates between yields and PR42, PRFS, NIC and CR42 tended to be lower in cows milked OAD than those milked TAD. Genetic correlations between MY, LY and PR42 in OAD cows were close to zero.

The genetic correlations of milk composition (FP, PP and LP) and all interval fertility traits were very low (OAD: -0.16 to 0.23; TAD: -0.06 to -0.31) in both milking populations. In cows milked TAD, the genetic correlations between milk composition and all interval fertility traits were negative and the genetic correlations of milk composition and binary fertility traits were positive. Compared with TAD milking cows, genetic correlations between FP, PP, LP, and binary fertility traits tended to be lower in OAD milking cows. Protein percentage was positively genetically correlated with SR21, SR42, PR21, PR42, PRFS, CR21 and CR42 in the TAD milking population (from +0.16 to +0.30). However, genetic correlations between PP and all binary fertility traits were lower (from -0.16 to +0.26) in the OAD milking population. The genetic correlations between LP and NIC or CR21 were negative and comparatively higher in OAD than TAD.

5.4.5 Correlations among the fertility traits

The phenotypic correlations among fertility traits were similar in both OAD and TAD milking populations. In both milking populations, phenotypic correlations between SMCO and FSCO were strongly positive. SMCO and FSCO were strongly positively phenotypically correlated with CI. Phenotypic correlations of SMFS and CFS with SR21 were strongly negative in both milking systems. Likewise, phenotypic correlations of SMCO, FSCO and CI with PR21, PR42, CR21 and CR42 were strongly negative in both milking populations. Strong positive phenotypic correlations of similar magnitudes were observed for PR21, PR42 with CR21 and CR42 in TAD and PR42 with CR21 and CR42 in OAD.

All fertility traits were strongly genetically correlated in cows milked TAD but the magnitudes of the genetic correlations varied from weak to strong in cows milked OAD. In the OAD milking cow population, genetic correlations between SMCO, FSCO, CI, PR21, PR42 and other binary fertility traits were strong. However, genetic correlations between fertility traits of time to first service (SMFS and CFS) and submission (SR21 and SR42) with other binary fertility traits were weak to moderate. The genetic correlations among the binary fertility traits were strong, but they were stronger in the TAD milking population. The genetic correlations between all interval traits and all binary traits were strong and negative, and these were stronger in the TAD milking cow population.

5.5 Discussion

5.5.1 Reproductive performance

This study has found that first parity cows milked TAD produced greater yields of milk, fat, protein and lactose than cows milked OAD. Fat and protein percentages were higher in OAD milking cows than TAD milking cows. These findings agree with the study by Lembeye et al. (2016), who also studied New Zealand dairy cows with a similar breed mix as the current study. The fertility outcomes of CI, SR21, PRFS and PR42 reported for TAD milking cows in current study are similar to the reported national averages for New Zealand dairy cows in the calving season from 2015-2016 to 2017-2018 (LIC and DairyNZ, 2021). The reproductive performance of cows milked OAD exceeded the reported national averages and is consistent with previous research that found that New Zealand cows milked in OAD herd had improved reproductive performance compared to those that were milked TAD (Hemming et al., 2018, Jayawardana et al., 2022). The better reproductive performance associated with OAD milking cows is hypothesized to be due to the reduced extent of negative energy balance (**NEB**) in early lactation in cows milked OAD (Phyn et al., 2014).

5.5.2 Variance components and heritability

The heritability estimates for MY, FY, PY, FP and PP in cows milked OAD obtained in this study are consistent with previously reported estimates by Lembeye et al. (2016), whose study population was multiparous OAD milking cows in New Zealand. Heritability estimates found in this study for MY, FY, and PY in cows milked TAD agree with other estimates in New Zealand dairy cattle (Sneddon et al., 2015; Lopez-Villalobos et al., 2020). The heritabilities for FP and PP in cows milked TAD are similar to those reported by Lembeye et al. (2016) in TAD milking cows. Heritability estimates for LP concur with previous findings by Lopez-Villalobos et al. (2020). The heritability estimates for yields (MY, FY, PY and LY) were greater in the OAD milking population compared with the estimates obtained in the TAD milking population. These findings, suggest that higher genetic responses for these traits could be obtained in the OAD milking population, when the response is measured in genetic standard deviations. However, when expressed in absolute values the genetic responses would be expected to be greater in the TAD milking population than the OAD population because the genetic variances are greater in the TAD milking population.

The heritability estimates for SMFS, SMCO, FSCO, CFS, CI, SR21, PR21, PR42 and CR42 ranged between 0.02 and 0.07 in cows milked TAD, in agreement with previously reported estimates in New Zealand (Grosshans et al., 1997; Harris et al., 2005; Pryce and Harris, 2006). Also, estimated heritabilities for fertility traits in this study are consistent with studies in Irish and Australian dairy cattle populations (Berry et al., 2013; Morton et al., 2018). In both milking systems heritability estimates for fertility traits were lower than heritability estimates for production traits. This suggests that the phenotypic expression fertility traits are more influenced by management and environmental factors than genetic factors. The estimated covariance components and heritabilities in this study can be used for future genetic evaluation studies and to develop separated selection indices for OAD and TAD dairy cattle populations in New Zealand.

5.5.3 Correlations between production traits

Overall, phenotypic and genetic correlations between production traits presented in Tables 5.4 and 5.5 are in line with previously reported estimates for New Zealand dairy cows milked in TAD and OAD populations (Ahlborn and Dempfle, 1992; Sneddon et al., 2015; Lembeye et al., 2016). The genetic correlations between FY and MY or PY in cows milked TAD in the current study are consistent with the findings of Lembeye et al. (2016). Greater genetic correlations were reported by Ahlborn and Dempfle (1992), and Sneddon et al. (2015) than our results (FY and MY:0.55-0.82, FY and PY: 0.69-0.88 and FY and LY:0.40). In agreement with the estimates reported by Costa et al. (2018), weak phenotypic and genetic correlations were observed between LP and FP or PP in this study (LP and FP: -0.02 and 0.01, LP and PP: -0.02 and -0.01, respectively). In both milking populations, most of the phenotypic and genetic correlations between milk yields and milk composition traits are similar in direction and magnitude.

5.5.4 Correlations between production and fertility traits

Estimates of genetic correlations between milk production and fertility traits obtained in this study were, in general, antagonistic in both milking populations.

These estimates suggest that increases of production yields (MY, FY, PY) will result in longer intervals of SMFS, SMCO, FSCO, CFS, CI, and decreases in SR21, SR42, PR21, PR42, PRFS, CR21 and CR42 in the TAD milking cow population. These unfavorable genetic correlations agree with most previous literature in New Zealand (Grosshans et al., 1997; Harris et al., 2005; Pryce and Harris, 2006), Ireland (Evans et al., 2002; Berry et al., 2003), Australia (Haile-Mariam et al., 2003; Morton et al., 2018) and the United Kingdom (Pryce et al., 1998). Though cows milked OAD had better phenotypic reproductive performances (Jayawardana et al., 2020), we found phenotypic associations between milk production and fertility traits were negligible. Further research is required to identify the causal factors that reduce phenotypic associations between milk production and fertility in both milking cow populations. The low phenotypic correlations between production and fertility traits suggest that still there is potential to reduce the negative impact of high milk production on reproductive performance through improving management practices in both milking cow populations.

The negative genetic correlations between yields and FSCO are interesting in the OAD milking population, suggesting that increasing genetic merit for milk production decreases the length of FSCO in cows milked OAD. The genetic correlations of MY and LY with SMCO, PRFS and PR42 were lower in cows milked OAD compared with cows milked TAD. These findings suggested that OAD milking cows with higher genetic merit for production had a shorter interval from SMCO, that is they are more likely to conceive to their first service and to get in-calf early in the breeding season than TAD milking cows. Furthermore, our results demonstrate the genetic correlation of MY and NIC is very low in cows milked OAD and phenotypic correlations of NIC with milk composition (FP, PP and LP) and production traits (MY, FY, PY and LY) were also close to zero. This indicates the levels of production and milk composition are not associated with overall pregnancy rate in OAD milking populations. Edwards (2018) reported that fewer OAD milking cows were removed from the herd due to being non-pregnant in New Zealand and more were removed due to the low production, udder health and mastitis. However, our results indicate the genetic correlations for yields and SMFS, SR21 and CR21 are more unfavorable in the OAD compared with TAD. Reasons for this unfavorable association have not been identified in this study. This is an area where further research is required.

At present, two binary traits of SR21, CR42 and calving season day are used for genetic evaluation of fertility in New Zealand dairy cows (Bowley et al., 2015). The genetic correlations observed for MY, FY, PY with SR21 in TAD milking cows agree with the estimates reported by Pryce and Harris (2006) for first parity cows in New Zealand (-0.23, -0.10 and -0.13, respectively). However, our estimates for the genetic correlations between MY, FY, PY and CR42 in cows milked TAD are slightly greater than the estimates by Pryce and Harris (2006) (-0.15, -0.06 and-0.05, respectively) and Harris et al. (2005) (MY and CR42: -0.16) for first parity cows. To our knowledge, this is the first study that estimates the genetic and phenotypic correlations between MY and NIC in the New Zealand dairy cattle population. A positive genetic association was found with MY and NIC in cows milked TAD, suggesting that high-producing cows milked in TAD are more likely to be not in-calf. Morton et al. (2018) reported a positive genetic correlation between 120-d MY and

NIC (0.23) in the Australian dairy cattle population, similar to our findings in TAD milking cows.

Results from the current study identified positive genetic associations of milk composition (FP, PP and LP) with binary traits (except NIC) and negative associations with interval fertility traits in cows milked TAD. Those cows producing milk with higher percentages of fat, protein, and lactose are more likely to have better fertility in TAD milking cow population. In agreement with our findings, Morton et al. (2018) reported positive genetic associations between 120-d PP and SR21, PR42, and PRFS (0.28, 0.28 and 0.23, respectively) and negative genetic associations for 120-d PP and CFS, CI and NIC (-0.32, -0.23, -0.17, respectively). The genetic correlations identified between LP, PP and CI agree with those reported by Haile-Mariam and Pryce (2017) (LP and CI: -0.23 and PP and CI: -0.28). Furthermore, Carty et al. (2020) reported LP measured at 0-30 DIM had the strongest relationship with SMCO in the Irish cattle population. The positive genetic associations observed for PP with SR21, PR21, PR42, PRFS, CR21 and CR42 in cows milked TAD suggest that genetic selection for increased PP would be expected to increase genetic merit for fertility in cows. Morton et al. (2017) suggested that the possible causes of the positive genetic association between reproductive traits and PP is due to selection for both high fertility and milk protein production.

The estimated genetic associations between PP and fertility traits in cows milked OAD differ from those in cows milked TAD. Many studies demonstrate that both milk composition and reproductive performance are influenced by early lactation energy balance of dairy cows (Friggens et al., 2007; Patton et al., 2007; van Knegsel et al., 2014). Benedet et al. (2020) reported early lactation blood BHB and NEFA were negatively genetically associated with PP and LP in Italian Holstein cows. Morton et al. (2016) reported that associations between PP and reproductive performance are probably partly due to factors other than extent of NEB in early lactation. Therefore, further research should be carried out to estimate correlations between variables which are contributing to NEB and PP in OAD milking population. Our results indicate LP is negatively genetically correlated with NIC and CR42 in the OAD milking population. Cows with low genetic merit for LP are more likely to be not in-calf and less likely to have calved by 6 wk in following season in the OAD milking population.

5.5.5 Correlations between fertility traits

The estimated genetic and phenotypic correlations between fertility traits in the TAD milking cow population agree with estimates reported by Grosshans et al. (1997). Correlations between SR21 and CR42 agree with findings by Harris et al. (2005) and Pryce and Harris (2006). Morton et al. (2018) reported genetic and phenotypic associations of PR42 with CFS, CI, SR21, PRFS and NIC for Australian dairy cows, values which are similar to those found in the TAD population in the current study.

Most of the phenotypic associations among fertility traits were consistent across the milking regimens. The reproductive management of OAD and TAD cows is similar, with most herds in New Zealand inseminating cows only once per day, most often in the morning. Mating periods on average are shorter in OAD herds (71.8 d vs. 76.1 d) (Hemming et al., 2018). The genetic and phenotypic associations among interval fertility traits were strongly positively correlated due in part to the whole relationships between them. We found most of genetic correlations among SMFS, CFS, SR21 and SR42 and other fertility traits were low to moderate in cows milked OAD whereas SMCO, FSCO, CI, PR21, PR42 and other binary fertility traits are strongly genetically correlated in cows milked OAD. These findings suggest that in OAD milking herds a cow's genetic merit for ability to conceive has greater impact on her phenotypic expression of fertility than the ability to show oestrus. The genetic correlations between fertility traits were stronger with low standard errors but not unity in cows milked TAD and genetic correlations of fertility traits were weak to strong with higher standard errors in cows milked OAD; therefore, in OAD herds, emphasis on several fertility traits will be necessary to achieve progress as correlated gains from emphasis on a single trait will be smaller than in TAD cows.

We found non-converged and unrealistic genetic correlation estimates of CR42 with CI, PRFS, NIC and CR21 in the OAD milking cow population. Cows milked OAD had higher 6-wk calving rate than TAD (89% vs 82%). The binary trait CR42 included few zero values, and this may be a reason for the non-converged algorithms in ASReml parameter estimation. Standard errors for genetic correlation estimates were relatively greater in cows milked OAD than cows milked TAD due to relatively less amount of data in OAD milking population. Amer et al. (2016) reported when including more traits in a multi-trait model, genetic parameters have remained stable but standard error increased substantially. Furthermore, Craig et al. (2018) reported that low heritabilities of the fertility traits and potential culling bias reflected high standard errors of the genetic correlation estimates, particularly for traits expressed in later parities.

Once daily milking is becoming a popular milking practice in New Zealand and about 31% of dairy farmers in Northland region adopted full season OAD milking (DairyNZ, 2022). In this context, investigation of phenotypic and genetic relationship is important for OAD milking population. The growing use of early-aged pregnancy testing allowed us to derive PR21, PR42 and NIC using early aged PD records, which is an accurate method of determining the conception date. In present analysis, cows that had missing AB records and conceptions were retained with penalised dates at the extreme end of the distribution in both milking cow populations. Previous studies in different dairy cattle populations also estimate the genetic correlations by assigning the penalty values for the cows that do not experience the event (Grosshans et al., 1997; Haile-Mariam et al., 2003; Morton et al., 2018). This approach introduces some bias, where some cows have an assigned value greater and some less than they would have achieved had they been allowed longer to display the trait. Nevertheless, to exclude these cows would have introduced a greater bias, in that the performance of the least fertile cows was not considered.

Since this is the first study to estimates genetic and phenotypic correlations for fertility traits for the OAD milking cow population, we do not have other estimates to compare with our findings. The current system of genetic evaluation for fertility traits do not separate the cows or sires according to milking regimens in New Zealand. The estimated genetic and phenotypic correlations in cows milked OAD can be used to develop fertility breeding values specifically for the OAD milking cow population and to develop separate selection indices for the OAD and TAD dairy cattle populations. Further research is required to evaluate the interaction between genotype by milking frequency for fertility traits in New Zealand and other countries adopting once daily milking systems.

5.6 Conclusions

In this study we found heritability estimates for milk composition and fertility traits were similar for cows that were milked either OAD or TAD in seasonal calving New Zealand dairy herds. The genetic and phenotypic associations between milk production traits were similar regardless of the milking regimen. Phenotypic correlations between milk production and fertility traits were negligible. However, genetic correlations between milk production and fertility were antagonistic in both milking populations. The genetic correlations between milk production and key reproductive metrics of PR42, NIC and CR42 were smaller in cows milked OAD than TAD. This implies that the difference in milking regimen or other management practices in OAD milking herds could have lessened the antagonistic genetic association between those traits. Milk protein concentration was positive genetically correlated with fertility traits in TAD milking cow population. This indicates that there is potential to increase the fertility performance of dairy cattle by identifying the mechanisms of the association between PP and fertility traits in cows milked TAD. Further studies are required to identify these associations for cows milked OAD. The present findings can be used to develop separate selection indexes for OAD milking population.

5.7 References

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Chapter 6

Identification of genomic regions associated with milk composition and fertility traits in spring-calved dairy cows in New Zealand

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6.1 Abstract

The objective of this study was to identify genomic regions and genes that are associated with milk composition and fertility traits of spring-calved dairy cows in New Zealand. The phenotypic data from 2014-2015 to 2021-2022 calving seasons in two Massey University dairy herds was used. We identified 73 SNPs that were significantly associated with 58 potential candidate genes for milk composition and fertility traits. Four SNPs on chromosome 14 were highly significant for both fat and protein percentages and associated genes were DGAT1, SLC52A2, CPSF1 and MROH1. For fertility traits, significant associations were detected for intervals from start of mating to first service, start of mating to conception, first service to conception, calving to first service and 6-wk submission, 6-wk in-calf, conception to the first service in the first 3-wk of the breeding season, not in-calf and 6-wk calving rates. Gene ontology revealed 10 candidate genes (KCNH5, HS6ST3, GLS, ENSBTAG00000051479, STAT1, STAT4, GPD2, SH3PXD2A, EVA1C and ARMH3) that were significantly associated with fertility traits. The biological functions of these genes are related to reduce the metabolic stress of cows and increase insulin secretion during the mating period, early embryonic development, fetal growth, and maternal lipid metabolism during the pregnancy period.

Keywords: Genome-wide association study, milk composition, fertility, dairy cattle, candidate gene

6.2 Introduction

The New Zealand dairy farming is pasture-based with cows calving in Winter (June/July) in each production season. To retain a seasonal calving pattern, dairy farmers aim to maximise the proportion of cows mated and in-calf early in the breeding season to generate more days in milk. In New Zealand, the most common breeds used in dairy farming are Holstein Friesian (F; 32.5%), Jersey (J; 8.2%) and crossbred of Holstein Friesian and Jersey (F × J; 49.6%) (LIC and DairyNZ, 2021). The milk production of New Zealand dairy cows has increased in recent production seasons, due to improvements in management practices and genetic selection for milk production (LIC and DairyNZ, 2021). Since 2001 cow fertility has been incorporated national genetic evaluation in New Zealand (Harris and Montgomerie, 2001; Harris et al., 2006). The New Zealand national averages for 3-wk submission (SR21), conception to first service (PRFS), 6-wk in-calf (PR42) were 81.3%, 52.7%, and 67.7% in 2020-2021 production season, respectively (LIC and DairyNZ, 2021). Kerslake et al. (2018) reported that reproductive failure is the primary cause of cow wastage in New Zealand dairy herds. About 31% of annual cost of wastage (NZ$7,279/100 cows) is associated with cow removals due to failure to conceive or maintain a pregnancy.

Heritability estimates for fertility traits are low in New Zealand dairy cows (<10%) and antagonistic genetic correlations have been reported between production and fertility traits in New Zealand dairy cows (Grosshans et al., 1997; Harris and Montgomerie, 2001; Jayawardana et al., 2023). The simultaneous improvement of milk production and fertility is difficult to achieve in dairy cattle breeding due to this antagonistic relationship. In this context, genomic selection can be used in dairy cattle breeding programs to improve the rate of genetic gain by increasing the accuracy of genomic breeding values (Goddard and Hayes, 2007; Hayes et al., 2009; Meuwissen et al., 2013) and reducing the generation intervals (Schaeffer, 2006). Genomic selection uses the information of genome-wide single nucleotide polymorphisms (SNPs) markers. Ma et al. (2019) reported that the development of genomic selection on fertility traits has stabilised and even reversed the declining trend of cow fertility.

Genome-wide association studies (GWAS) are a powerful tool for detecting genomic regions that explain the genetic variation of phenotypic traits. Identification of fertility-associated quantitative trait loci (QTL) would support the efficiency of genomic selection for fertility traits. Previous studies have identified several QTL regions and functional candidate genes associated with milk composition and fertility traits of dairy cows in several countries (Boichard et al., 2003; Berry et al., 2012; Höglund et al., 2014; Nayeri et al., 2016; Lu et al., 2020). Since 2008, genomically enhanced breeding values have been used for the genomic selection in New Zealand dairy cattle (Harris et al., 2008; Winkelman et al., 2015).

In New Zealand, cows that produce milk with more fat and protein are more profitable for dairy farmers because the payment system rewards yields of fat and protein and penalises milk volume. It is important to identify the genomic regions and genes that are associated with fat and protein percentages to improve the genomic breeding values in those traits. The most important measures of fertility performance of dairy cows are SR21, PR42 and not in-calf rate (NIC) at end of the breeding season. However, no published GWAS were found in the literature reporting candidate genes associated with SR21, PR42 and NIC. There is little information available on GWAS for fertility traits in spring-calved dairy cows in New Zealand. Though the fertility performance of New Zealand dairy cattle is higher than the Irish, Australian, United Kingdom and USA dairy cattle populations (Jayawardana et al., 2022), it is well below New Zealand industry targets (SR21=90%, PRFS=60% and PR42=78% (Burke and Fowler, 2007), and potentially the existing fertility breeding values could be enhanced with the use of genomic information.

The objective of the present study was to identify the SNPs and candidate genes affecting milk composition and fertility traits in spring-calved dairy cows milked in Massey University dairy herds in New Zealand.

6.3 Materials and methods

6.3.1 Data

Data from Massey University Dairy 1 and Dairy 4 herds, Palmerston North, New Zealand were used for this study. Calving, mating, pregnancy diagnosis, herd testing milking dates, lactation yields and pedigree records of spring-calved dairy cows were extracted from the Livestock Improvement Corporation (LIC), New Zealand. The initial dataset consists of 6,931 records of 2,270 cows from 2014-2015 to 2021-2022 calving seasons. Both herds had at least three herd testing milk records per lactation during the study period. Breed distribution was 129 Holstein Friesian (F), 117 Jersey (J) and 239 crossbred of Holstein Friesian and Jersey (F × J) cows in Dairy 1 and 430 F, 23 J and 696 F × J cows in Dairy 4. The management of cows at Dairy 1 includes full-lactation once-daily milking with minimal supplementary feeding, and a low stocking density (2.1 cows/ha). In contrast, the Dairy 4 herd is milked twice daily, with higher levels of supplementary feeding and a greater stocking density (2.8 cows/ha). Data from calving season 2014-2015 in Dairy 1 herd was excluded from the present analysis because it was the transitional year of changing from milking twice daily to once daily.

6.3.2 Phenotypic traits

Milk composition and fertility traits investigated in this study are presented in Table 6.1. Fat percentage (FP) and protein percentage (PP) and lactose percentage (LP) was calculated for each lactation record as the ratio between fat yield, protein yield or lactose yield and milk yield multiplied by 100.

Table 6.1. Description of phenotypic (milk composition and fertility) traits used in this study

Trait	Criteria for trait calculation
Milk composition	
FP	Fat percentage, kg fat per kg milk (%)
PP	Protein percentage, kg protein per kg milk (%)
LP	Lactose percentage, kg lactose per kg milk (%)
Fertility	
SMFS[1]	The interval from the start of mating to the first service (d)
SMCO[2]	The interval from the start of mating to conception (d)
FSCO[2]	The interval from the first service to conception (d)
CFS[1]	Interval from calving to the first service (d)
CI[3]	Interval between two consecutive calvings (d)
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)
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)
PRFS	Cows conceived to the first AB insemination in the first 21 d from the start of mating date were represented as 1, otherwise coded as 0 (binary)
NIC	Cows not in-calf at the end of the mating period represented as 1 and in calf cows were coded as 0 (binary)
CR21[4]	Cows calved in the first 21 d from the planned start of calving date represented as 1, otherwise coded as 0 (binary)
CR42[4]	Cows calved in the first 42 d from the planned start of calving date represented as 1, otherwise coded as 0 (binary)

[1] Intervals for cows with no recorded artificial breeding (AB) were ended at the end of the AB period of herd in each calving season (n=29).

[2] Intervals for cows that did not conceive were ended at the herd's end of the mating date plus 21 days (n=787).

[3] Intervals for cows with positive pregnancy diagnosis results and without subsequent calving dates were calculated by estimating a calving date using conception date plus 282 days (n=1113).

[4] Cows that did not calve in the subsequent season were treated as missing variables and both traits were not calculated for 2021-2022 calving season.

The mating start date and mating end date for each calving season were sourced from the LIC database. Cows (n=161) with mating records outside the mating season in Dairy 4 herds in the calving season of 2018-2019 were excluded for fertility traits calculation. Those cows were used for other experiments that demanded another mating strategy.

The average mating length of the breeding season was 71.0 d in Dairy 1 and 69.5 d for Dairy 4. Cows (n=328) that were missing both a pregnancy diagnosis (PD) result and all herd tests after the end of the breeding period were assumed to have left the herd prior to the end of the mating period and were excluded from the analysis. Pregnancy diagnosis results where the foetal age was estimated and where cows were tested between 35-122 d (inclusive) from conception were used. Conception dates were calculated as the date of PD minus the estimated foetal age for cows with a pregnancy status of 'pregnant'. In some cases, cows (n=63) with positive PD had estimated pregnancy day counts outside the bounds (<35 d or >122 d) or no estimated fetal age results but calved in the subsequent season. In such cases conception dates were calculated as subsequent calving dates minus gestation length of 282 d. Conception date was not calculated for cows (n=16) with pregnancy status as 'pregnant' but without either estimated fetal age or subsequent calving date. These cows were culled due to mastitis, low production and udder problems.

Cows (n=29) with no recorded artificial breeding (AB) inseminations were retained with a penalty date in the analysis. The penalty date was assigned for first service date as the end of the AB period in each herd within the calving season. The end of the AB period was defined for each herd as the date of the last AB insemination that was not followed by another AB insemination within 7 d (DairyNZ, 2019). Likewise, cows that had not conceived (n=787) at the end of the breeding season were included, with a penalty date for conception as the mating end date in each herd plus 21 d (Johnston and Bunter, 1996; Grosshans et al., 1997). Calving interval (CI) was calculated for cows that did not calve in the subsequent calving season but had a positive pregnancy diagnosis (n=1,113). The calving date was assigned as conception date plus gestation length of 282 d and CI was calculated as the assigned calving date minus the calving date in the respective season.

Submission in the first 21 d (SR21) or 42 d (SR42) of mating season was coded as 1 if a cow that had at least one AB record in the first 21 d or 42 d from the start of the mating date, respectively, and 0 otherwise. Pregnant by 21 d (PR21) or 42 d (PR42) was coded as 1 if a cow was pregnant in the first 21 d or 42 d of from the mating start date, respectively, otherwise 0. Only cows whose first service was to AB and within first 21 days from the mating start date were used to determine pregnant to first service (PRFS), which was classified as 1 for cows whose date of first service coincided with their date of conception, and 0 otherwise. Not in-calf (NIC) at end of the breeding season was coded as 1 for cows with the last PD outcome ‘empty’ and 0 for cows with last PD outcome ‘pregnant’. Cows (n=18) with last pregnancy status ‘doubtful’ without subsequent calving were also coded as 1. Planned start of calving (PSC) date was calculated for a herd by adding 282 d to the herd’s mating start date in each calving season. If a cow calved less than 21 d or 42 d after the PSC then, 3-wk calving (CR21) or 6-wk calving (CR42) was coded as 1, otherwise 0.

The final phenotypic data set comprised 6,382 records. Five parity classes were defined, cows in their first four parities were considered separately and parity number five or higher were grouped into one class. The 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.

6.3.3 Descriptive statistics

Descriptive statistics for milk production and fertility traits were obtained using the MEANS procedure of SAS package 9.4 (SAS Institute Inc. 2013, Cary, NC, USA).

6.3.4 Genotypes and quality control

DNA was extracted from ear punch tissue samples for genotyping with the Illumina Bovine Illumina 50K SNP-chips. Initial genotype data consisted of 1,774 cows with 132,154 SNPs during the study period. The SNP & Variation Suite (SVS 8.8) software was used for quality control. The genotypes recorded in Illumina A/B allele format were converted to 0, 1 or 2, depending on the number of B alleles present at each locus. In the filtering process firstly, SNPs with a call rate ≤ 80% were excluded. Then individuals with a call rate ≤ 80% or with a minor allele frequency (MAF) ≤ 5% and significant deviation from Hardy-Weinberg Equilibrium (HWE) P values ($P < 10^{-6}$) were excluded from the data set. After the quality control steps, a total of 1,537 individuals with 42,667 SNPs were selected for association analysis.

6.3.5 Genome-wide association analyses

Phenotypes used for the GWAS were pre-corrected using fixed effects, covariates and random effects using the ASReml 4.1 software package (Gilmour et al., 2015). A single-trait animal repeatability model was used to adjust repeated measures in the phenotypes. The models were included the fixed effects of herd-year as a contemporary group, parity (1, 2, 3, 4, ≥5), the regression coefficient associated with proportion of F, the regression coefficient associated with heterosis, and the regression coefficient associated with deviation of calving date from median calving date of the herd within season as a covariate and random effect of animal, cow permanent environment effect and random residual.

A genome-wide association analysis (GWAS) using a mixed linear model was used to test associations between individual SNP traits using GCTA software (Yang et al., 2011).

The following model was fitted for each trait,

$$y = \mu + X\beta + g + e$$

where y was the pre-corrected phenotype for the cow, μ was overall mean, β was the fixed effect of the candidate SNP to be tested for the association, X was the SNP genotype indicator variable coded as 0, 1 or 2, g was the random effect that captures polygenic effect of other SNPs with $g \sim N(0, \mathbf{G}\sigma_g^2)$, where **G** was the genomic relationship matrix between cows and σ_g^2 was the additive genetic variance explained by markers and e was the random residual effect with $e \sim N(0, \mathbf{I}\sigma_e^2)$, where **I** was the identity matrix of oder n= 1,537, and σ_e^2 was the residual variance. The variance of y was assumed as $\mathrm{var}(\mathbf{y}) = \mathbf{G}\sigma_g^2 + \mathbf{I}\sigma_e^2$. Diagonal and off-diagonal values of the **G** were calculated as follows,

$$G_{jk} = \frac{1}{m}\sum_i G_{ijk}$$

$$= \begin{cases} 1 + \frac{1}{m}\sum_{i=1}^{m} \frac{x_{ij}^2 - (1+2p_i)x_{ij} + 2p_i^2}{2p_i(1-p_i)}, & j = k \\ \frac{1}{m}\sum_{i=1}^{m} \frac{(x_{ij}-2p_i)(x_{ik}-2p_i)}{2p_i(1-p_i)}, & j \neq k \end{cases}$$

where G_{ijk} was the estimated genomic relationship between animal j and k at locus i, and m is total number of SNPs (m=42,667), x_{ij} and x_{ik} were genotypes with the number of copies for reference of copies for reference allele for the i^{th} SNP j^{th} and k^{th} animal and p_i was the allele frequency of the allele for which the homozygous genotype was coded as 2.

The estimated associations were represented in Manhattan plots in which −log10(p-value) were plotted against the genomic locations of the markers using qqman package in R software 4.2.1(Turner, 2014). The significance threshold values for the GWAS were estimated using a Bonferroni multiple-test correction, which adjusts p-values due to the increased risk of a type I error when making multiple statistical tests (Armstrong, 2014). Bonferroni-corrected genome-wide significance threshold was estimated as 0.05/m ($0.05/42{,}667 = 1.17 \times 10^{-6}$), which was 5.93 on a −log10 (p-value) scale. The Bonferroni correction has been considered too conservative, therefore, suggestive significance threshold was also estimated as 1/m. The p-value threshold for suggestive associations was 2.34×10^{-5}(1/42,667), which corresponded to 4.63 in −log10(p-value) scale. The genomic position, allele substitution effect and their standard errors, and closest gene were described for the SNPs that exceed the Bonferroni adjusted p-value thresholds.

6.3.6 Candidate genes and functional analysis

Potential candidate genes were explored using the Ensembl (release 107) (http://www.ensembl.org/index.html) based on the Bos taurus reference genome in ARS-UCD1.2 genome assembly (Yates et al., 2019). The individual significant SNP for each trait were examined to locate the closest genes within 100 kb upstream and downstream from the identified SNP. Gene annotation was recorded if the gene was positioned 5 kb upstream or downstream from gene boundaries. The biological functions of the associated candidate genes were reviewed using the Gene Ontology (GO) tool in Ensembl.

6.4 Results

6.4.1 Descriptive statistics

Descriptive statistics for milk composition and fertility traits of selected cow population are presented in Table 6.2.

Table 6.2. Descriptive statistics of milk composition and fertility traits of cows at Massey Dairy 1 and Dairy 4

Trait[1]	No.records	Mean	SD	CV	Min.	Max.
Yields						
Milk	6381	4969	1237	24.9	387	8861
Fat	6381	232	51	21.9	22	398
Protein	6381	188	42	22.2	19	311
Lactose	6381	251	63	25.1	11	449
Milk composition						
FP%	6381	4.75	0.69	14.5	2.92	7.38
PP%	6381	3.83	0.32	8.3	2.96	5.14
LP%	6381	5.04	0.18	3.6	2.84	5.48
Fertility						
SMFS	6221	10.9	8.6	79.1	1	71
SMCO	6221	29.6	28.4	95.8	1	110
FSCO	6192	19.8	27.7	139.7	1	109
CFS	6221	79.6	18.0	22.6	14.0	201
CI	5434	368.7	21.9	5.9	261	435
SR21(%)	6221	91.4	28.0	30.7	0	1
SR42(%)	6221	99.2	8.8	8.8	0	1
PR21(%)	6221	54.1	49.8	92.0	0	1
PR42(%)	6221	75.9	42.8	56.4	0	1
PRFS(%)	6219	47.8	49.9	104.4	0	1
NIC(%)	6381	12.2	32.7	268.4	0	1
CR21(%)	4321	68.0	46.6	68.6	0	1
CR42(%)	4321	91.0	28.6	31.4	0	1

[1] FP = fat percentage; PP = protein percentage; LP = lactose percentage; SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); CI = consecutive calving interval (d); SR21 = cow inseminated in the first 3 weeks from the start of mating; SR42 = cow inseminated in the first 6 weeks from the start of mating; PR21 = cow conceived in the first 3 weeks from the start of mating; PR42 = cow conceived in the first 6 weeks from the start of mating; PRFS = cow conceived to first service in the first 3 weeks of the breeding season; NIC = cow not in calf at end of the breeding season; CR21 = cow calved in the first 3 weeks from the planned start of the calving; CR42 = cow calved in the first 6 weeks from the planned start of the calving.

Cows produced on average 4,968 L of milk yield, 232 kg of fat yield, 188 kg of protein yield and 251 kg of lactose yield during the study period. Average intervals for first AB and conception after the herd's start of the mating dates were 10.9 days and 29.6 days, respectively. A higher proportion of cows were mated within the first 3 weeks and 6 weeks of the mating season (91% and 99%, respectively). Fifty-four percent and 76% of cows conceived in the first 3 weeks and 6 weeks of the mating seasons, respectively and 48% were pregnant to their first service. Not in-calf at end of breeding seasons was 12%. Calving by first 3 weeks and 6 weeks from the PSC was 68% and 91%, respectively.

6.4.2 GWAS for milk composition traits

Figure 6.1 shows the Manhattan plots for fat, protein and lactose percentages. The SNPs that surpassed the genome-wide significance p-value threshold ($P < 1.17\times10^{-6}$) with position, allele substitution effect, gene annotation, candidate gene name and description are presented in Table 6.3.

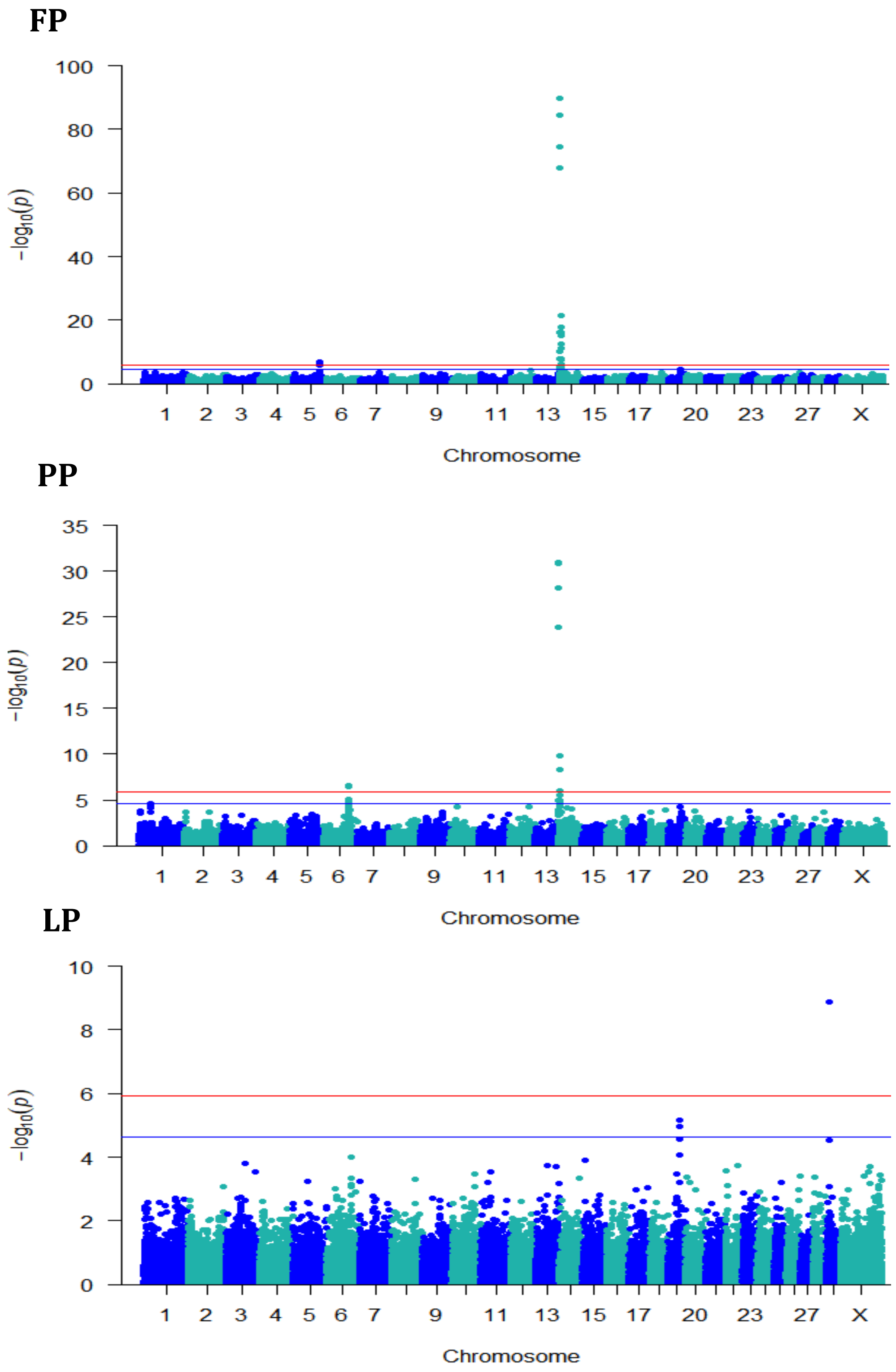


Figure 6.1. Manhatton plots for -log10 *p*-values of markers effects for milk composition traits of fat percentage (**FP**), protein percentage (**PP**) and lactose percentage (**LP**). Genome-wide significance threshold of Bonferroni correction is represented in red line at $-\log_{10}$ (*p*-value) = 5.93 and suggestive significance threshold is represented in blue line at $-\log_{10}$ (*p*-value) = 4.63.

Table 6.3. The genome-wide significant single nucleotide polymorphisms (SNPs) for milk composition traits in dairy cattle

Trait	Locus	Chr	Position	$-\log_{10}(P)$	Effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
FP	rs211210569	5	93,945,738	6.76	0.11 (0.02)	C	T	0.62	Intron	MGST1	microsomal glutathione S-transferase 1
	rs210744919	5	93,949,810	6.43	-0.11 (0.02)	G	A	0.41	Intron	MGST1	microsomal glutathione S-transferase 1
	rs110984572	14	1,653,693	7.85	-0.17 (0.03)	C	T	0.89	Upstream	FOXH1	forkhead box H1
	rs134432442	14	1,736,599	74.54	-0.33 (0.02)	C	T	0.52	Missense	CPSF1	cleavage and polyadenylation specific factor 1
	rs211309638	14	1,757,935	10.01	-0.20 (0.03)	C	T	0.89	Upstream	ADCK5	aarF domain containing kinase 5
	rs137071126	14	1,765,835	84.41	-0.36 (0.02)	C	G	0.48	Synonymous	SLC52A2	solute carrier family 52 member 2
	rs109421300	14	1,801,116	89.79	-0.38 (0.02)	T	C	0.46	Intron	DGAT1	diacylglycerol O-acyltransferase 1
	rs137787931	14	1,880,378	67.95	0.32 (0.02)	T	C	0.55	Intron	MROH1	maestro heat like repeat family member 1
	rs109742607	14	2,217,163	16.15	0.16 (0.02)	A	G	0.69	Intron	IQANK1	IQ motif and ankyrin repeat containing 1
	rs110323635	14	2,239,085	16.15	-0.16 (0.02)	A	G	0.31	missense	MAPK15	mitogen-activated protein kinase 15
	rs109617015	14	2,386,688	17.86	-0.22 (0.03)	A	G	0.16	Intron	ZC3H3	zinc finger CCCH-type containing 3
	rs109529219	14	2,468,020	21.47	-0.22 (0.02)	G	A	0.22	Intron	RHPN1	rhophilin Rho GTPase binding protein 1
	rs109958270	14	2,605,493	7.81	0.12 (0.02)	C	T	0.78	intergenic	-	-
	rs110043428	14	2,790,501	12.49	0.14 (0.02)	A	G	0.53	intergenic	-	-
	rs109476486	14	2,826,632	12.41	-0.17 (0.02)	T	G	0.20	upstream	LYPD2	LY6/PLAUR domain containing 2
	rs110545978	14	3,186,141	15.28	0.18 (0.02)	T	C	0.83	intergenic	-	-
	rs136880486	14	4,078,923	11.13	-0.13 (0.02)	T	C	0.28	upstream	AGO2	argonaute RISC catalytic component 2
	rs110755656	14	5,274,635	7.65	0.11 (0.02)	G	T	0.76	intergenic	-	-
	rs110359329	14	7,428,315	6.09	-0.09 (0.02)	A	G	0.58	intergenic	-	-

Table 6.3. The genome-wide significant single nucleotide polymorphisms (SNPs) for milk composition traits in dairy cattle (continued)

Trait	Locus	Chr	Position	$-\log_{10}(P)$	Effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
PP	rs43703015	6	87,390,576	6.52	0.05 (0.01)	T	C	0.61	Missense	CSN3	casein kappa
	rs43703016	6	8,7390,612	6.52	0.05 (0.01)	C	A	0.61	Missense	CSN3	casein kappa
	rs110014544	6	87,390,673	6.52	0.05 (0.01)	G	A	0.61	synonymous	CSN3	casein kappa
	rs109787476	6	87,390,681	6.43	0.05 (0.01)	T	A	0.61	3 prime UTR	CSN3	casein kappa
	rs134432442	14	1,736,599	28.13	-0.09 (0.01)	C	T	0.52	Missense	CPSF1	cleavage and polyadenylation specific factor 1
	rs137071126	14	1,765,835	30.83	-0.10 (0.01)	C	G	0.48	synonymous	SLC52A2	solute carrier family 52 member 2
	rs109421300	14	1,801,116	30.96	-0.10 (0.01)	T	C	0.46	Intron	DGAT1	diacylglycerol O-acyltransferase 1
	rs137787931	14	1,880,378	23.91	0.09 (0.01)	T	C	0.55	Intron	MROH1	maestro heat like repeat family member 1
	rs109742607	14	2,217,163	6.59	0.04 (0.01)	A	G	0.69	Intron	IQANK1	IQ motif and ankyrin repeat containing 1
	rs109617015	14	2,386,688	8.31	-0.07 (0.01)	A	G	0.16	Intron	ZC3H3	zinc finger CCCH-type containing 3
	rs109529219	14	2,468,020	9.81	-0.07 (0.01)	G	A	0.22	Intron	RHPN1	rhophilin Rho GTPase binding protein 1
	rs110545978	14	3,186,141	8.33	0.06 (0.01)	T	C	0.83	Intergenic	-	-
	rs110755656	14	5,274,635	5.94	0.05 (0.01)	G	T	0.76	Intergenic	-	-
LP	rs378183369	29	9,563,396	8.89	-0.03 (0.004)	A	G	0.74	Intron	PICALM	phosphatidylinositol binding clathrin assembly protein

Chr = chromosome; SE = standard error; Ref. = reference allele; MA= minor allele; Ref. freq. = reference allele frequency; FP = fat percentage; PP = protein percentage.

A total of 33 SNPs met the genome wide significance threshold were across the traits FP, PP and LP. According to the results, 19 SNPs from chromosomes 5 and 14 were significantly associated with FP, 13 SNPs from chromosomes 6 and 14 were significantly associated with PP and one SNP on chromosome 29 was significantly associated with LP at genome-wide significance threshold. The majority of associations were found on chromosome 14 for both FP and PP. The four top SNPs on chromosome 14 (14:1,736,599, 14:1,765,835, 14:1,801,116 and 14:1,880,378) were highly significant for both FP and PP, with genome wide significance levels of -log10 (P) = 74.5, 84.4, 89.8 and 68.0 for FP, 28.1, 30.8, 31.0 and 23.9 for PP.

The associated SNP markers were annotated to 26 potential candidate genes for FP, PP and LP at a genome wide significance level. For both FP and PP, the majority of significant SNPs were mapped to introns (39%), followed by the intergenic regions (21%), missense variants (15%), upstream regions closest to the candidate genes (12%), synonymous variants (9%) and 3 prime UTR (3%). The highly significant peak on chromosome 14 mapped to the DGAT1 gene for FP and PP. The four most significant SNPs for both FP and PP that are located on chromosome 14 are introducing a missense variant in the CPSF1 gene, a synonymous variant in the SLC52A2 gene and, intron of the DGAT1 and MROH1 genes, respectively. Likewise, three significant SNPs on chromosome 14 are located within the intron of the IQANK1, ZC3H3 and RHPN1 genes for both FP and PP. The SNPs that are positioned on 14: 1,653,693, 14: 1,757,935, 14: 2,826,632 and 14: 4,078,923 were annotated upstream of genes FOXH1, ADCK5, LYPD2 and AGO2, respectively.

Two significant SNPs (5: 93,945,738, 5: 93,949,810) on chromosome 5 for FP were found in the MGST1 gene, located within an intron region of the MGST1 gene.

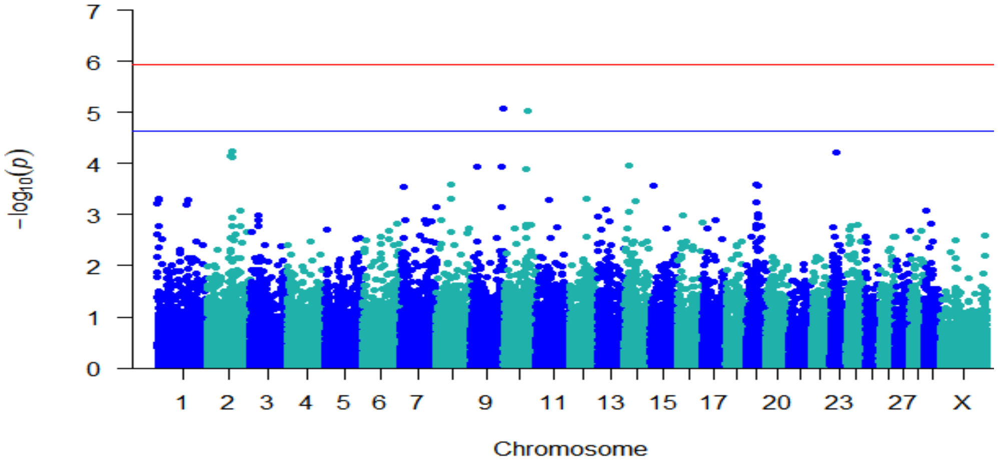
Likewise, four significantly associated SNPs (6:87,390,576, 6:8,7390,612, 6:87,390,673 and 6:87,390,681) on chromosome 6 for PP mapped to CSN3 and those are located missense, synonymous and 3 prime UTR of the CSN3 gene.

Although there were strong peaks on chromosome 14 for both FP and PP, none of the SNPs on chromosome 14 was not associated with LP. The single SNP was detected genome widely significant for LP on chromosome 29 and located within the intron of the gene PICALM. Also, one peak on chromosome 19 was observed and two SNPs on this region were suggestively significant. The SNPs that surpassed the suggestive significance p-value threshold ($P < 2.34 \times 10^{-5}$) are explained in Annexure 1.

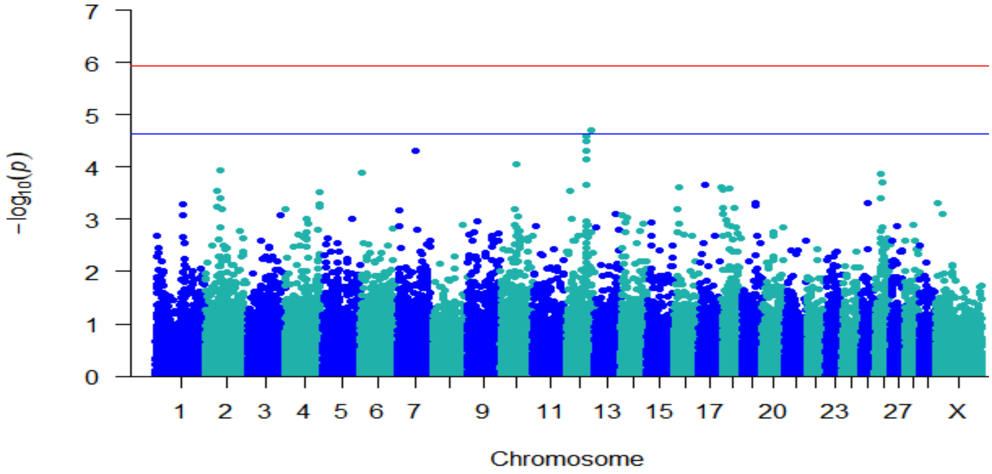
6.4.3 GWAS for fertility traits

The Manhattan plots from the GWAS with fertility traits are shown in Figure 6.2. The SNPs that surpassed both genome-wide and suggestive significance levels for studied fertility traits are presented in Table 6.4 with the position, allele substitution effect, gene annotation and candidate gene name.

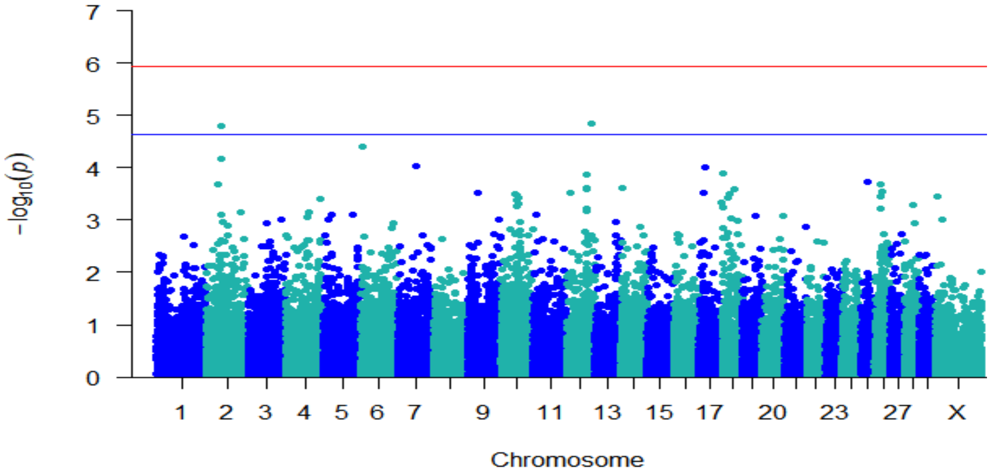
SMFS



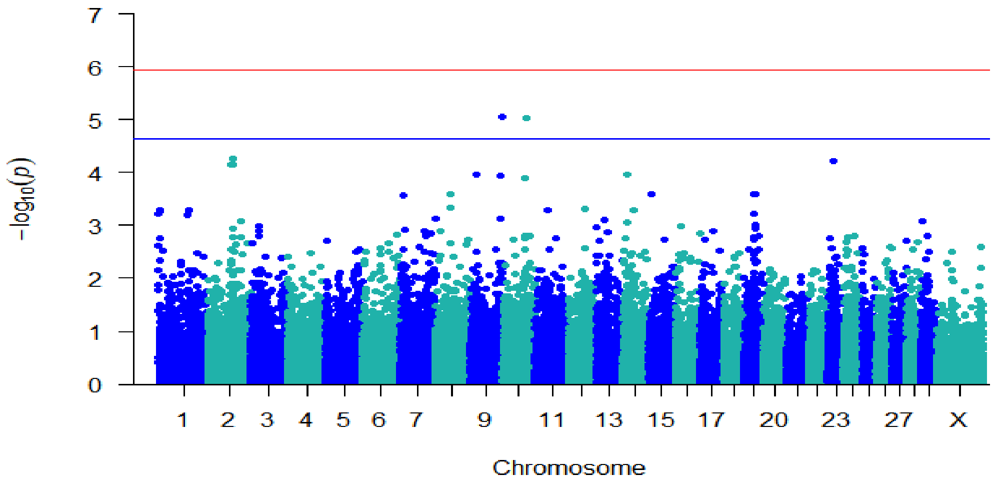
SMCO



FSCO



CFS


−log10(p)
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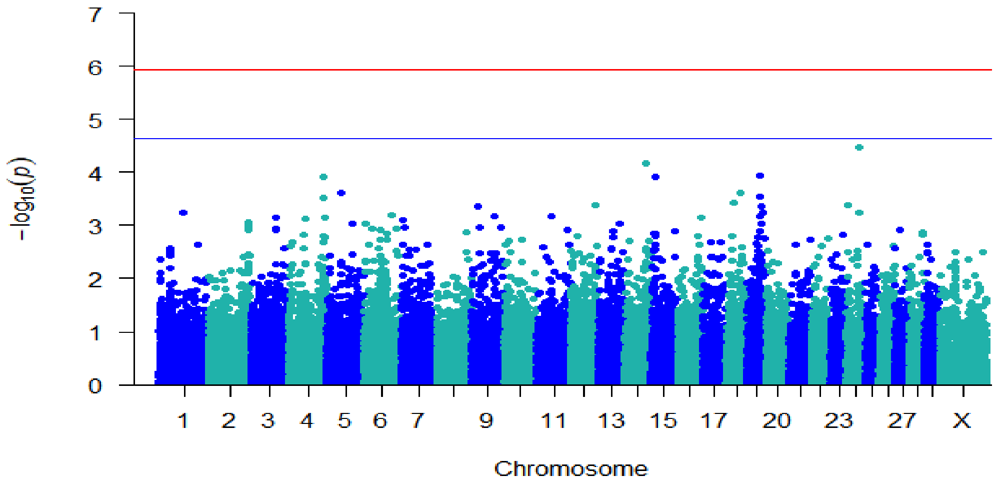
X
Chromosome

CI


−log10(p)
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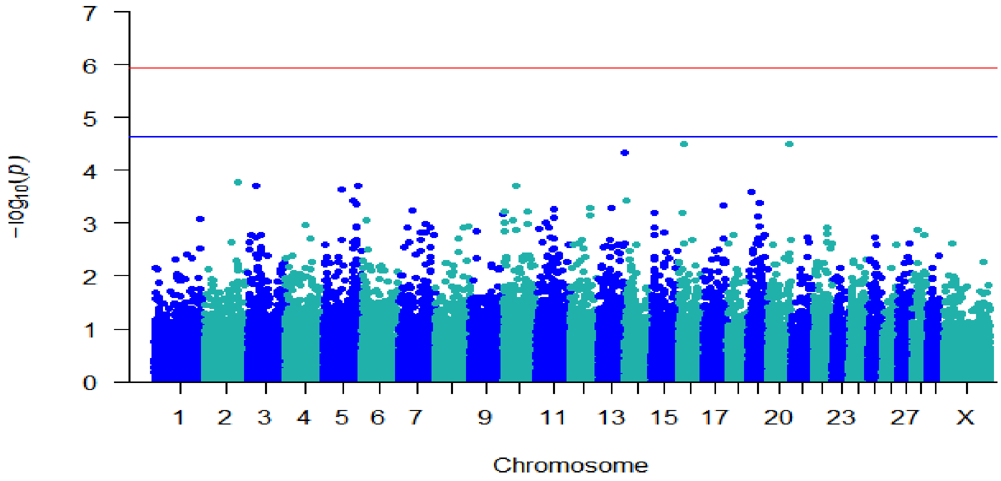
23
27
X
Chromosome

SR21


−log10(p)
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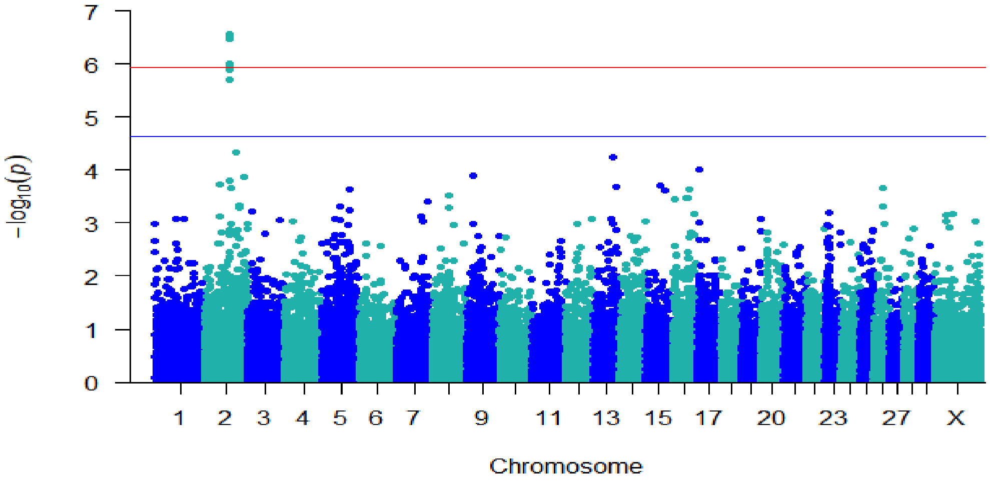
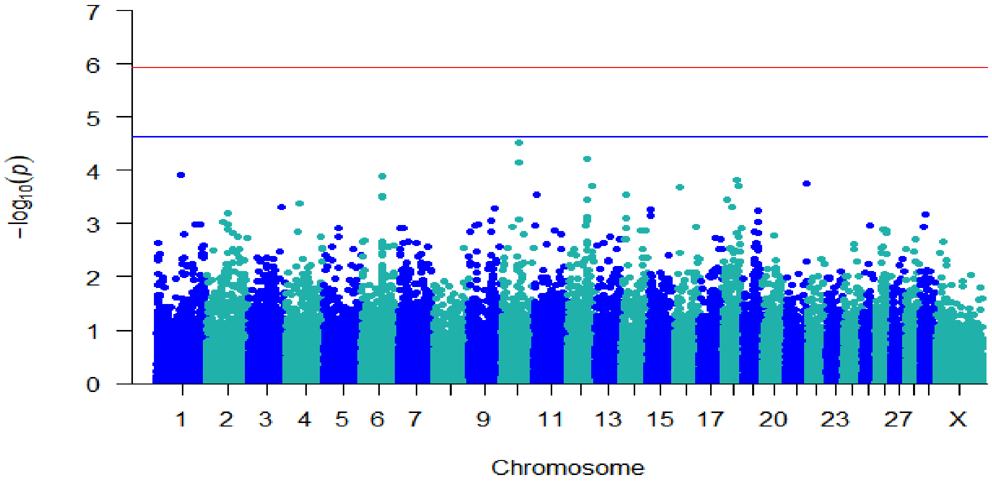
X
Chromosome

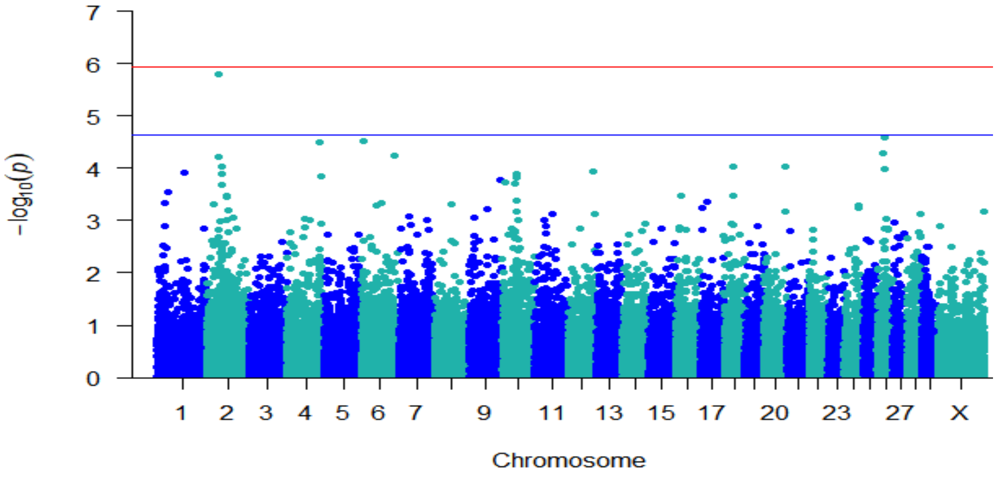
SR42



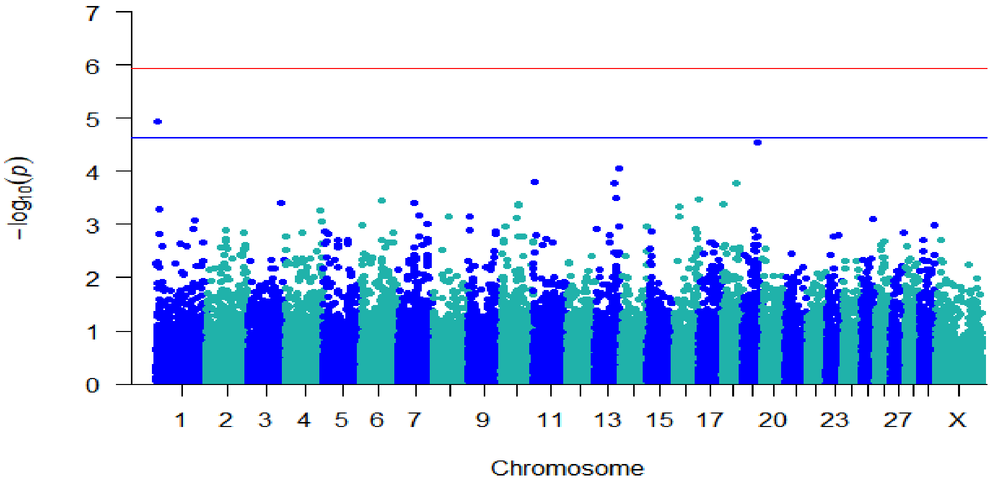
PR21



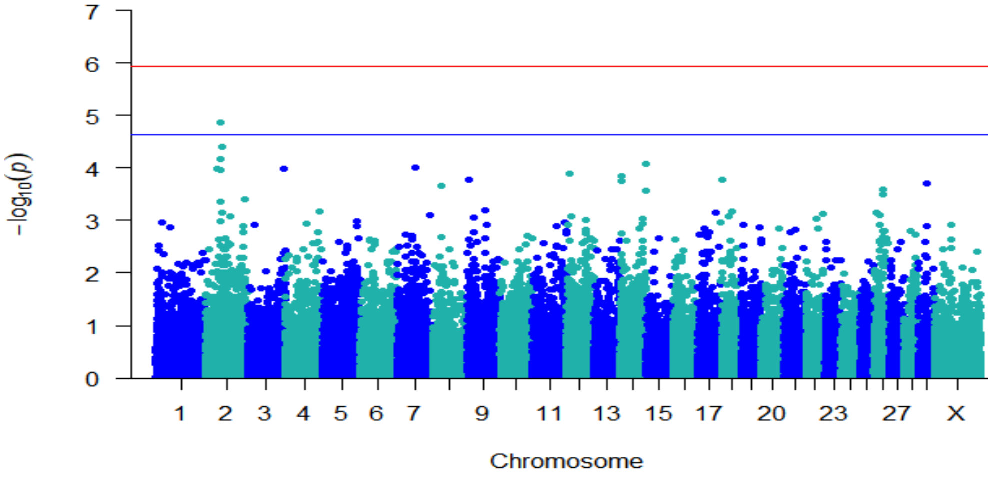
PR42



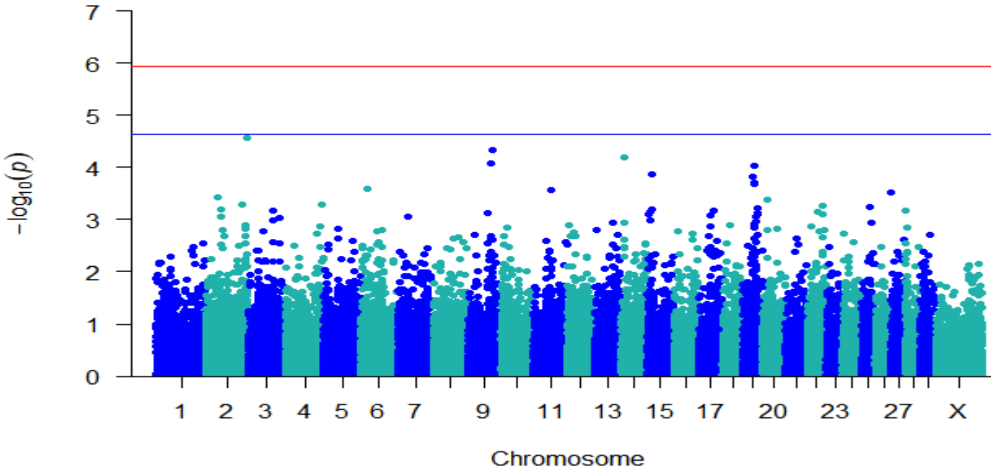
PRFS



NIC



CR21



CR42

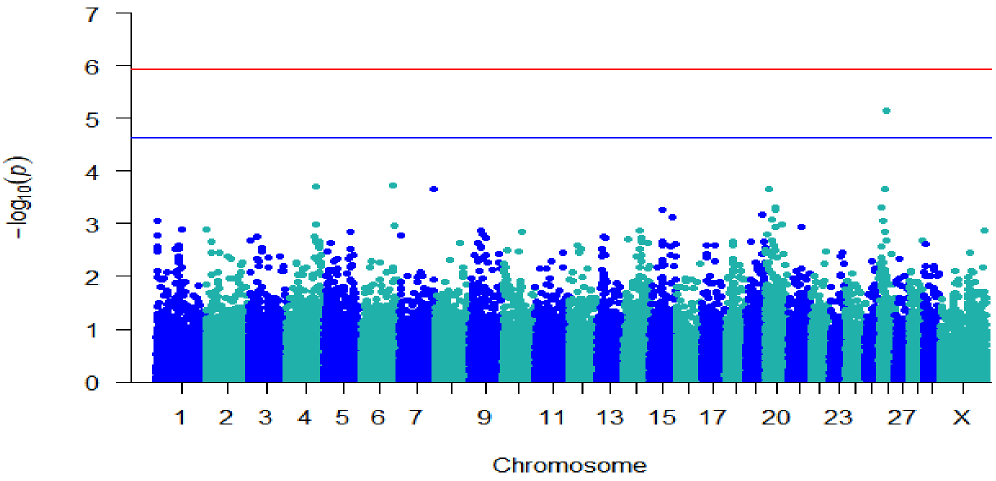


Figure 6.2. Manhattan plots for -log10 *p*-values of markers effects for the interval from start of mating to the first service (SMFS), interval from the start of mating to conception (SMCO), interval from the first service to conception (FSCO), interval from calving to the first service (CFS), consecutive calving intervals (CI), 3 weeks submission (SR21), 6 weeks submission (SR42), 3 weeks in-calf (PR21), 6 weeks in-calf (PR42), pregnant to the first service in the first 3 weeks of the breeding season (PRFS), not in-calf at end of the breeding season (NIC), 3 weeks calving (CR21) and 6 weeks calving rate (CR42). Genome-wide significance threshold of Bonferroni correction is represented in red line at $-\log_{10}$ (*p*-value) = 5.93 and suggestive significance threshold is represented in blue line at $-\log_{10}$ (*p*-value) = 4.63.

Table 6.4. The single nucleotide polymorphisms (SNPs) that identified as significance at genome-wide and suggestive significance thresholds for the fertility traits[1]

Trait	Locus	Chr	Position	$-\log_{10}(P)$	SNP effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
SMFS	rs132976072	9	101,238,301	5.08	0.06 (0.01)	A	G	0.48	intergenic	-	-
	rs110111959	10	75,105,774	5.02	0.07 (0.02)	A	G	0.75	intron	KCNH5	potassium voltage-gated channel subfamily H member 5
SMCO	rs41635004	12	77,611,452	4.71	1.52×10^{-7} (3.55×10^{-8})	A	G	0.63	intron	HS6ST3	heparan sulfate 6-O-sulfotransferase 3
FSCO	rs132906739	2	48,960,169	4.81	-0.05 (0.01)	A	G	0.57	intergenic	-	-
	rs109941542	2	49,095,661	4.81	-0.05 (0.01)	C	T	0.57	intergenic	-	-
	rs41635004	12	77,611,452	4.85	0.04 (0.01)	A	G	0.63	intron	HS6ST3	heparan sulfate 6-O-sulfotransferase 3
CFS	rs132976072	9	101,238,301	5.06	0.06 (0.01)	A	G	0.48	intergenic	-	-
	rs110111959	10	75,105,774	5.02	0.07 (0.02)	A	G	0.75	intron	KCNH5	potassium voltage-gated channel subfamily H member 5
SR42	rs135632251	2	79,410,669	5.71	0.001(0.0002)	C	T	0.94	intergenic	-	-
	rs134983646	2	79,474,217	5.95	-0.0005 (9.82×10^{-5})	G	A	0.19	intron	GLS	glutaminase
										ENSBTAG00000051479	-
	rs109798660	2	79,486,672	6.47	-0.001 (9.95×10^{-5})	T	C	0.19	intron	ENSBTAG00000051479	-
	rs134911740	2	79,706,385	5.99	-0.0005 (9.81×10^{-5})	G	A	0.19	intergenic	-	-
	rs135975975	2	79,817,588	6.52	-0.001 (9.94×10^{-5})	G	A	0.18	intron	GLS	Glutaminase

Table 6.4. The single nucleotide polymorphisms (SNPs) that identified as significance at genome-wide and suggestive significance thresholds for the fertility traits[1] (continued)

Trait	Locus	Chr	Position	$-\log_{10}(P)$	SNP effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
SR42	rs137812009	2	79,908,334	6.50	-0.001 (9.90×10^{-5})	T	C	0.18	intron	STAT1	signal transducer and activator of transcription 1
	rs41610299	2	79,946,595	6.57	-0.001 (9.93×10^{-5})	T	C	0.18	intron	STAT4	signal transducer and activator of transcription 4
	rs380321634	2	79,975,164	5.89	0.001(0.0002)	T	G	0.94	intron	STAT4	signal transducer and activator of transcription 4
PR42	rs137030801	2	39,681,141	5.80	4.20×10^{-9} (8.72×10^{-10})	T	C	0.46	intron	GPD2	glycerol-3-phosphate dehydrogenase 2
	rs109673037	26	24,432,758	4.74	0.002 (0.0004)	C	T	0.42	upstream	SH3PXD2A	SH3 and PX domains 2A
PRFS	rs132685083	1	2,262,097	4.93	0.004 (0.001)	A	G	0.45	intron	EVA1C	eva-1 homolog C
NIC	rs132906739	2	48,960,169	4.86	-3.06×10^{-8} (7.04×10^{-9})	A	G	0.57	intergenic	-	-
	rs109941542	2	49,095,661	4.86	-3.06×10^{-8} (7.04×10^{-9})	C	T	0.57	intergenic	-	-
CR42	rs41606045	26	22,526,369	5.14	0.003 (7.32×10^{-6})	C	T	0.85	intron	ARMH3	armadillo like helical domain containing 3

Chr = chromosome; SE = standard error; Ref. = reference allele; MA= minor allele; Ref. freq. = reference allele frequency.

[1] SMFS = start of mating to first service (d); SMCO = start of mating to conception (d); FSCO = first service to conception (d); CFS = calving to the first service (d); SR42 = cow inseminated in the first 6 weeks from the start of mating; PR21 = cow conceived in the first 3 weeks from the start of mating; PR42 = cow conceived in the first 6 weeks from the start of mating; PRFS = cow conceived to first service in the first 3 weeks from start of mating; NIC = cow not in calf at end of the breeding season.

Genome-wide significant associations were found only with SR42. Seven genome-wide significant SNPs (2:79,474,217, 2:79,486,672, 2:79,706,385, 2:79,817,588, 2:79,908,334, 2:79,946,595, 2:79,975,164) were detected on chromosome 2 for SR42, which are located in intron variants within the genes GLS, ENSBTAG00000051479, STAT1, STAT4. In total 22 SNPs were found to be associated with SMFS, SMCO, FSCO, CFS, SR42, PR42, PRFS, NIC and CR42 at suggestive significant threshold and the most significant SNPs for fertility traits were detected on chromosome 2. Association analysis did not detect any significant SNP for the traits CI, SR21, PR21 and CR21 either at genome-wide significance or suggestive significance levels.

The significantly associated SNPs were annotated to 10 potential candidate genes on chromosomes 1, 2, 9, 10, 12 and 26. Most of the suggestive significant SNPs for fertility traits were mapped to introns (59%), 36% to intergenic regions and the remaining 5% were located upstream to closest to the genes. The individual SNP 10:75,105,774 was significantly associated with SMFS and CFS and located within intron of the gene KCNH5. Likewise, the SNP at position 12:77,611,452 was significantly associated with both of SMCO and FSCO and mapped within the intron of the gene HS6ST3. Two SNPs on chromosome 2 (2: 39,681,141) and 26 (26: 24,432,758) were detected as significant for PR42, which are positioned within the intron of the gene GPD2 and upstream of the gene SH3PXD2A, respectively. One significant SNP (1: 2,262,097) was identified for PRFS and which is located within the intron of the gene EVA1C. Two significant SNPs for NIC on chromosome 2 were annotated as intergenic variants. The SNP at position 26: 22,526,369 was significantly associated with CR42 and which is located within the intron of the candidate gene ARMH3.

6.5 Discussion

The present study investigated the genome-wide associations and candidate genes for milk composition and fertility traits using 42,667 SNPs from 1,537 spring-calved dairy cows in two New Zealand herds. Cows milked in both Massey dairy herds had superior fertility performance compared with national averages for New Zealand dairy cows in the calving season from 2014-2015 to 2021-2022 (LIC and DairyNZ, 2021) (SR21=78.1-81.3%; PRFS=52.4-54.2%; PR42=65.8-67.8%). Our results of SMFS, SR21, PR21, and PR42 are in agreement with the findings by Rodriguez-Cutzal et al. (2022), who reported the fertility traits of SMFS (6.5-11.3 d), SMCO (15.2-21.6 d), SR21(92-98%), PR21(48-68%) and PR42 (74-89%) for production seasons 2016 and 2017 in Massey University Dairy 1 and Dairy 4 herds. For both milk composition and fertility traits, we identified 40 SNPs and 73 SNPs that were significantly associated with 32 and 58 potential candidate genes at genome-wide and suggestive significant levels, respectively in this population.

6.5.1 GWAS for milk composition traits

A very significant SNP associated with the diacylglycerol O-acyltransferase 1 (DGAT1) gene for both FP and PP in this study is widely reported with milk yield and composition traits in several dairy cattle populations (Pryce et al., 2010; Meredith et al., 2012; Buitenhuis et al., 2014; Raven et al., 2014). The DGAT1 gene was reported to be involved in biological functions of monoacylglycerol biosynthetic process (GO:0006640), triglyceride biosynthetic process (GO:0019432), lipid storage (GO:0019915), very-low-density lipoprotein particle assembly (GO:0034379), long-chain fatty-acyl-CoA metabolic process (GO:0035336), retinol metabolic process (GO:0042572), diacylglycerol metabolic process (GO:0046339), glycerolipid metabolic process (GO:0046486) and fatty acid homeostasis (GO:0055089). The DGAT1 gene plays an important role in triacylglycerol synthesis by catalyzing the formation of an ester linkage between a fatty acyl-CoA and the free hydroxyl group of diacylglycerol (Yen et al., 2008), triacylglycerols are the major constituent of milk fat (Pacheco-Pappenheim et al., 2019).

Also, we identified three SNPs that are close to DGAT1 and significantly associated with FP and PP in this population. The associated candidate genes are CPSF1, SLC52A2 and MROH1. The genes CPSF1 and SLC52A2 are involved in mRNA polyadenylation (GO:0006378) and riboflavin transport (GO:0032218), respectively. Riboflavin is essential for the metabolism of fats and proteins. The strong association found between a riboflavin transporter gene and FP and PP suggests that the genetic control of riboflavin content is likely related to plasma transport rather than to factors related to microbial metabolism in the rumen. The biological function of MROH1 gene is undiscovered, the association with FP and PP was identified in previous studies (Sanchez et al., 2017; Wang et al., 2020). These four candidate genes (DGAT1, CPSF1, SLC52A2 and MROH) have been previously reported as peak variants for fourier-transformed mid-infrared wavenumbers with highly significant protein sequence association effects for FP and PP in New Zealand dairy cattle (Tiplady et al., 2021).

Other candidate genes identified for FP and PP in this population were IQANK1, ZC3H3 and RHPN1. The gene IQANK1 is related to the regulation of barbed-end actin filament capping (GO:2000812), this gene is a neighbor gene of DGAT1 and has been shown to have causal effects on milk production traits independent of linkage disequilibrium (Xiang et al., 2022). The gene ZC3H3 is involved mRNA 3'-end processing (GO:0031124), positive regulation of activin receptor signaling pathway (GO:0032927) and regulation of mRNA polyadenylation (GO:1900363). Whilst RHPN1 codes negative regulation of stress fibre assembly (GO:0051497). Similar to our findings Oliveira et al. (2018) reported that genes ZC3H3 and RHPN1 were significantly associated with milk yield in Canadian Holstein and Jersey cattle. We found seven genes (DGAT1, CPSF1, SLC52A2, MROH, IQANK1, ZC3H3 and RHPN1) that are significantly associated with both FP and PP in this population. This suggests that genes that influence multiple traits are likely to be the main source of genetic correlations between traits. The results of SNP marker-trait associations in present study corroborate the strong positive genetic correlation (+0.72) between FP and PP in New Zealand dairy cows (Lembeye et al., 2016).

We found four significant SNPs on chromosome 14 for FP that are located upstream of genes FOXH1, ADCK5, LYPD2 and AGO2. Associations of these genes with FP have not been identified in the New Zealand dairy cow population previously. The FOXH1 gene is a member of the family Forkhead box (Fox) O1, which is a primary transcription factor in glucose metabolism, in the regulation of hepatic lipid metabolism (Sparks and Dong, 2009). The association of FOXH1 with milk fatty acids composition, fat yield and FP has been well documented in previous studies (Nayeri et al., 2016; Cruz et al., 2019; Atashi et al., 2020; Bohlouli et al., 2022). The biological functions of gene ADCK5 and LYPD2 are unknown, but, Ibeagha-Awemu et al. (2016) reported that candidate gene ADCK5 was associated with FP in Canadian Holstein cows whereas LYPD2 gene was associated fat yield of polish Holstein Friesian bulls (Frąszczak and Szyda, 2016). The biological functions of AGO2 are mostly related to the microRNAs (miRNAs) processing (GO:0035196) and miRNA metabolic process (GO:0010586), which regulates gene expression. A genome-wide association study by Freitas et al. (2020) has shown that AGO2 is associated with short-chain, medium-chain, long-chain, saturated, and unsaturated milk fatty acid groups in North American Holstein cattle. The candidate genes CPSF1, SLC52A2, MROH, IQANK1, ZC3H3, RHPN1, FOXH1, ADCK5, LYPD2 and AGO2 found on chromosome 14 in this study could be used as a basis of linkage disequilibrium studies in future to test whether any of these genes that neighbour DGAT1 are associated with variation in milk fat percentage of New Zealand dairy cattle, and to test the candidate status of DGAT1.

The MGST1 gene on chromosome 5 is associated with FP and has biological functions related to glutathione transport (GO:0034635), cellular oxidant detoxification (GO:0098869) and cellular response to lipid hydroperoxide (GO:0071449), which is the highly reactive primary oxygenated products of polyunsaturated fatty acids. The functional relationship of this gene with FP has not previously been identified in New Zealand dairy cattle, however, an association study by Lopdell et al. (2017) reported that MGST1 is a strong candidate gene for lactose yield in New Zealand dairy cows. The CSN3 associated with PP is in the casein gene family and improves milk protein quality and cheese production. This gene has biological functions related to regulating milk secretion from the mammary glands

(GO:0007595) and protein stabilization (GO:0050821). We identified three SNPs on chromosomes 19 and 29 that were associated with LP. This supports the previous association studies in New Zealand (Lopdell et al., 2017; Tiplady et al., 2021). The most significant SNP was in the intron of the PICALM gene on chromosome 29 for LP and biological functions mostly code endocytosis activity (GO:0006897), receptor-mediated endocytosis (GO:0006898) and regulation of endocytosis (GO:0030100). The functional relationship of the PICALM gene with milk protein content and cheesemaking properties has been previously reported in French dairy cows (Sanchez et al., 2018; Tribout et al., 2020).

6.5.2 GWAS for fertility traits

We focused on both interval and binary fertility traits for association analysis in the present study since minimising the intervals from the start of mating to the first service and the start of mating to conception are key drivers of reproductive outcomes in seasonal calving systems. We found that the candidate gene KCNH5 is associated with SMFS and CFS in this population, which has not been previously reported with these two traits. The KCNH5 codes ion transport (GO:0006811), potassium ion transport (GO:0006813) and potassium ion transmembrane transport (GO:0071805). Potassium is an essential micromineral component for the reproduction of early lactation dairy cows to counter metabolic acid load during heat stress (NRC, 2001).

Our results indicate that the candidate gene HS6ST3 is associated with SMCO and FSCO. The biological function of HS6ST3 is related to heparan sulfate proteoglycan biosynthetic process (GO:0015012) and heparan sulfate proteoglycan biosynthetic process with enzymatic modification (GO:0015012). Previous studies on heparan sulfate biosynthesis have revealed that heparan sulfate proteoglycans and their binding proteins play a critical role in embryonic development and growth factors (Ringvall et al., 2000; Kirn-Safran et al., 2008; Sarrazin et al., 2011). Itoh and Sokol (1994) demonstrated that heparan sulfate proteoglycans participate in gastrulation and mesoderm formation in the early embryo. Thus, associations of this gene with early-stage conception-related traits of cows support the findings of improved fertility performances in this population. No previous study has identified the association of this gene with fertility traits of dairy cows, but associations have been reported with this gene and fat yield of Italian and Swiss Brown Swiss dairy cows (Prinsen et al., 2017), fatty acids composition (Jiang et al., 2011; Ahmad et al., 2022) and mastitis resistance traits in Holstein dairy cows (Siebert, 2017).

Our results identified that four genes (GLS, ENSBTAG00000051479, STAT1 and STAT4) located on chromosome 2 were associated with SR42. However, none of those genes were associated with SR21. We do not have an explanation for this finding. The biological functions of GLS and ENSBTAG00000051479 are similar and related to glutamate biosynthetic processes (GO:0006537), glutamine metabolic processes (GO:0006541) and glutamine catabolic processes (GO:0006543). Glutamine is synethesised from glutamate, via glutamine synthetase (Watford, 2015). Glutamine occupies a central role linking energy and protein metabolism (Meijer et al., 1993). Glutamine and glutamic acid are the most abundant amino acids in milk protein (Meijer et al., 1993). Infusion of glutamine has also been shown to modulate immune response (Caroprese et al., 2012). Metabolic stress in early lactation has been identified as a risk factor associated with a delayed return to ovarian cyclicity and decreased fertility of dairy cows (Butler and Smith, 1989; Kessel et al., 2008). While a direct mechanism linking glutamine with reproductive outcomes has not been identified we know that glutamine plays a critical role as a signaling molecule in amino acid- and glucose-stimulated insulin secretion and enhancing sensitivity to insulin (Li et al., 2004; Newsholme et al., 2007). The early lactation period of dairy cattle is characterised by severe negative energy balance, with lower blood glucose and insulin concentrations and higher blood GH concentrations (Butler et al., 2003). We hypothesize that GLS and ENSBTAG00000051479 are promoting increased insulin concentrations and potentially reducing the negative energy balance of dairy cows during early lactation.

Previous literature links STAT family genes with reproductive outcomes in dairy cattle (Khatib et al., 2009; Walker et al., 2012; Ratcliffe et al., 2017). STAT1 and STAT4 are two genes of the STAT family that code for signal transduction (GO:0007165), regulation of transcription activity (GO:0006355) and cytokine-mediated signaling pathways (GO:0019221). STAT are the main drivers to growth factors, cytokines and hormones involved in reproductive processes (Levy and Darnell, 2002). STAT1 plays a key role activating the JAK/STAT signaling pathway, which regulates early embryonic development of dairy cows (Walker et al., 2012). STAT genes are involved in the regulation of implantation, establishing uterine receptivity and regulation of the maternal immune response during pregnancy (Maj and Chelmonska-Soyta, 2007). Khatib et al. (2009) revealed that STAT1 was related to fertilization and early embryonic survival rates in Holstein cattle. Associations of

STAT1 and STAT4 with milk production traits were also reported in Holstein dairy cows (Cobanoglu et al., 2006; Zhang et al., 2010). Further investigations with a larger dataset are warranted to determine whether any of these genes are associated with pregnancy related traits (PR21, PR42 and PRFS) in dairy cows in seasonal calving systems.

Two candidate genes, GPD2 and SH3PXD2A located on chromosomes 2 and 26 were associated with PR42 in this population. The gene GPD2, Glycerol-3-Phosphate Dehydrogenase 2 catalyzes the conversion of glycerol-3-phosphate to dihydroxyacetone phosphate, which esterified with fatty acids to form triglycerides (Sadri et al., 2011). This gene is associated with fatty acid and triglyceride synthesis in early lactation dairy cows (Graber et al., 2010; Sadri et al., 2011). The gene SH3PXD2A codes in utero embryonic development (GO:0001701), the process whose specific outcome is the progression of the embryo through the uterus over time, from the formation of the zygote in the oviduct, to the birth of calves. Associations of GPD2 and SH3PXD2A with PR42 have not been identified in previous GWAS for fertility traits, however, Palombo et al. (2018) reported that SH3PXD2A was associated with milk fatty acid composition in Italian Holstein dairy cows.

A single gene, EVA1C was associated with PRFS in this study. The biological function of this gene is yet unknown. We identified one candidate gene, ARMH3, for the trait CR42. The biological function of ARMH3 is related to the regulation of Golgi organization (GO:1903358) and functions of the Golgi apparatus are the transport, sorting and modification of both proteins and lipids (Teasdale and Jackson, 1996).

The most significant SNPs for fertility traits in the current study were located on chromosome 2, in agreement with previously reported GWAS for fertility traits

(Schulman et al., 2011; Berry et al., 2012; Höglund et al., 2015; Tenghe et al., 2016). However, the candidate genes found to be associated with fertility traits show little agreement with the previously reported GWAS (Minozzi et al., 2013; Parker Gaddis et al., 2016). Fertility traits are polygenic in nature and also influenced by non-genetic factors, for example, the heat detection ability of the farmer. The studies reported by Minozzi et.al (2013) and Parker Gaddis et al. (2016) involved indoor year-round calving in Italy and US dairy cattle, whereas this study was carried out with spring-calving cows under grazing conditions.

No significant SNPs were associated with CI, SR21, PR21 and CR21. A larger study population would increase the power to detect significant effects associated with fertility. Although many associations were detected with milk compositional traits, associations with fertility traits are limited in this population. Fertility traits have low heritability (<10%) and the low contribution of individual QTL to the total phenotypic variance also could be attributed to the low detection of associations for fertility traits in this study. Furthermore, many significant associations detected with fertility traits were associated at suggestive significance threshold in this study, which, on average, includes one false positive result across the genome. Lander and Kruglyak (1995) documented that suggestive linkage facilitated in reporting the promising but unproven findings that were worth in reporting for complex traits. In the present GWAS, cows were genotyped using medium density SNP panel (50K Illumina) and the study population consists of J, F and their crosses, however, the studied population consists of multi-breeds animals, which is a limitation of this study. The use of a high-density SNP panel would allow less reliance on linkage disequilibrium span and structure.

6.6 Conclusions

Genome-wide association analysis in the present study detected several regions and candidate genes associated with FP, PP, LP, SMFS, SMCO, FSCO, CFS, SR42, PR42, PRFS, NIC and CR42. Genomic regions and genes associated with milk composition traits FP, PP and LP in this research had been identified in previous studies. We identified several genes, (KCNH5, HS6ST3, GLS, ENSBTAG00000051479, STAT1, STAT4, GPD2 and SH3PXD2A) that were significantly associated with fertility outcomes in this population. The findings of this study provide an important foundation for future genome-wide association and fine-mapping studies for fertility traits in New Zealand dairy cattle. There findings should be validated in a larger population size before they could be applied to the genomic selection of fertility traits in New Zealand dairy cattle.

6.7 References

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Annexure 1: The suggestive significant single nucleotide polymorphisms (SNPs) for milk composition traits in dairy cattle

Trait	Locus	Chr	Position	$-\log_{10}(P)$	Effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
FP	rs134637616	5	93,945,655	5.74	0.11 (0.02)	G	T	0.66	intron	MGST1	microsomal glutathione S-transferase 1
	rs211210569	5	93,945,738	6.76	0.11 (0.02)	C	T	0.62	intron	MGST1	microsomal glutathione S-transferase 1
	rs210744919	5	93,949,810	6.43	-0.11 (0.02)	G	A	0.41	intron	MGST1	microsomal glutathione S-transferase 1
	rs41630614	14	1,514,056	5.09	0.09 (0.02)	T	G	0.68	upstream	ZNF7	zinc finger protein 7
	rs110984572	14	1,653,693	7.85	-0.17 (0.03)	C	T	0.89	upstream	FOXH1	forkhead box H1
	rs134432442	14	1,736,599	74.54	-0.33 (0.02)	C	T	0.52	missense	CPSF1	cleavage and polyadenylation specific factor 1
	rs211309638	14	1,757,935	10.01	-0.20 (0.03)	C	T	0.89	upstream	ADCK5	aarF domain containing kinase 5
	rs137071126	14	1,765,835	84.41	-0.36 (0.02)	C	G	0.48	synonymous	SLC52A2	solute carrier family 52 member 2
	rs109421300	14	1,801,116	89.79	-0.38 (0.02)	T	C	0.46	intron	DGAT1	diacylglycerol O-acyltransferase 1
	rs137787931	14	1,880,378	67.95	0.32 (0.02)	T	C	0.55	intron	MROH1	maestro heat like repeat family member 1
	rs109742607	14	2,217,163	16.15	0.16 (0.02)	A	G	0.69	intron	IQANK1	IQ motif and ankyrin repeat containing 1
	rs110323635	14	2,239,085	16.15	-0.16 (0.02)	A	G	0.31	missense	MAPK15	mitogen-activated protein kinase 15
	rs109617015	14	2,386,688	17.86	-0.22 (0.03)	A	G	0.16	intron	ZC3H3	zinc finger CCCH-type containing 3
	rs109529219	14	2,468,020	21.47	-0.22 (0.02)	G	A	0.22	intron	RHPN1	rhophilin Rho GTPase binding protein 1
	rs110060785	14	2,553,525	4.91	0.10 (0.02)	C	T	0.67	upstream	GPIHBP1	glycosylphosphatidylinositol anchored high density lipoprotein binding protein 1
	rs109958270	14	2,605,493	7.81	0.12 (0.02)	C	T	0.78	intergenic	-	-
	rs110626984	14	2,674,264	5.40	-0.11 (0.02)	T	C	0.30	intron	SLC45A4	solute carrier family 45 member 4
	rs110043428	14	2,790,501	12.49	0.14 (0.02)	A	G	0.53	intergenic	-	-
	rs109476486	14	2,826,632	12.41	-0.17 (0.02)	T	G	0.20	upstream	LYPD2	LY6/PLAUR domain containing 2
	rs134312854	14	2,898,515	4.95	-0.09 (0.02)	C	A	0.44	downstream	ARC	activity regulated cytoskeleton associated protein
	rs110545978	14	3,186,141	15.28	0.18 (0.02)	T	C	0.83	intergenic	-	-
	rs109289626	14	3,433,372	5.78	-0.11 (0.02)	A	G	0.43	intron	TRAPPC9	trafficking protein particle complex subunit 9
	rs110461662	14	3,765,019	5.22	-0.09 (0.02)	G	A	0.58	intron	DENND3	DENN domain containing 3
	rs136880486	14	4,078,923	11.13	-0.13 (0.02)	T	C	0.28	upstream	AGO2	argonaute RISC catalytic component 2
	rs110017379	14	4,364,952	5.33	-0.09 (0.02)	T	C	0.56	intron	TRAPPC9	trafficking protein particle complex subunit 9

Annexure 1: The suggestive significant single nucleotide polymorphisms (SNPs) for milk composition traits in dairy cattle (continued)

Trait	Locus	Chr	Position	$-\log_{10}(P$	Effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
FP	rs109917734	14	4,398,699	5.60	-0.10 (0.02)	G	A	0.50	intron	TRAPPC9	trafficking protein particle complex subunit 9
	rs109086264	14	4,414,829	4.65	0.08 (0.02)	A	C	0.40	intron	TRAPPC9	trafficking protein particle complex subunit 9
	rs110755656	14	5,274,635	7.65	0.11 (0.02)	G	T	0.76	intergenic	-	-
	rs110359329	14	7,428,315	6.09	-0.09 (0.02)	A	G	0.58	intergenic	-	-
	rs109396809	19	42,493,794	4.64	0.41 (0.08)	C	T	0.41	downstream	U6	U6 spliceosomal RNA
PP	rs43703015	6	87,390,576	6.52	0.05 (0.01)	T	C	0.61	missense	CSN3	casein kappa
	rs43703016	6	8,7390,612	6.52	0.05 (0.01)	C	A	0.61	missense	CSN3	casein kappa
	rs110014544	6	87,390,673	6.52	0.05 (0.01)	G	A	0.61	synonymous	CSN3	casein kappa
	rs109787476	6	87,390,681	6.43	0.05 (0.01)	T	A	0.61	3 prime UTR	CSN3	casein kappa
	rs110398459	6	87,733,586	4.78	0.05 (0.01)	G	T	0.26	intron	ENAM	Enamelin
	rs110398459	6	87,743,848	5.03	-0.04 (0.01)	A	G	0.38	intron	ENAM	Enamelin
	rs134432442	14	1,736,599	28.13	-0.09 (0.01)	C	T	0.52	missense	CPSF1	cleavage and polyadenylation specific factor 1
	rs137071126	14	1,765,835	30.83	-0.10 (0.01)	C	G	0.48	synonymous	SLC52A2	solute carrier family 52 member 2
	rs109421300	14	1,801,116	30.96	-0.10 (0.01)	T	C	0.46	intron	DGAT1	diacylglycerol O-acyltransferase 1
	rs137787931	14	1,880,378	23.91	0.09 (0.01)	T	C	0.55	intron	MROH1	maestro heat like repeat family member 1
	rs109742607	14	2,217,163	6.59	0.04 (0.01)	A	G	0.69	intron	IQANK1	IQ motif and ankyrin repeat containing 1
	rs110323635	14	2,239,085	4.96	-0.04 (0.01)	A	G	0.31	missense	MAPK15	mitogen-activated protein kinase 15
	rs109617015	14	2,386,688	8.31	-0.07 (0.01)	A	G	0.16	intron	ZC3H3	zinc finger CCCH-type containing 3
	rs109529219	14	2,468,020	9.81	-0.07 (0.01)	G	A	0.22	intron	RHPN1	rhophilin Rho GTPase binding protein 1
	rs110545978	14	3,186,141	8.33	0.06 (0.01)	T	C	0.83	intergenic	-	-
	rs136880486	14	4,078,923	5.58	-0.05 (0.01)	T	C	0.28	upstream	AGO2	argonaute RISC catalytic component 2
	rs110755656	14	5,274,635	5.94	0.05 (0.01)	G	T	0.76	intergenic	-	-
	rs109841945	14	6,276,199	4.74	-0.04 (0.01)	C	T	0.54	intergenic	-	-

Annexure 1: The suggestive significant single nucleotide polymorphisms (SNPs) for milk composition traits in dairy cattle (continued)

Trait	Locus	Chr	Position	$-\log_{10}(P$	Effect (SE)	Ref.	MA	Ref. freq.	Annotation	Gene name	Gene description
LP	rs137513051	19	42,932,520	5.16	-0.03 (0.01)	T	C	0.89	intron	KCNH4	potassium voltage-gated channel subfamily H member 4
	rs136271679	19	42,988,287	4.95	-0.03 (0.01)	C	T	0.89	intron	STAT5B	signal transducer and activator of transcription 5B
	rs378183369	29	9,563,396	8.89	-0.03 (0.004)	A	G	0.74	intron	PICALM	phosphatidylinositol binding clathrin assembly protein

Chr = chromosome; SE = standard error; Ref. = reference allele; MA= minor allele; Ref. freq. = reference allele frequency; FP = fat percentage; PP = protein percentage.

Chapter 7

General Discussion

7.1 Introduction

Seasonally concentrated calving with pasture-grazed dairy cows is characteristic of New Zealand dairy farming and fertility is one of the determining factors to achieve a sustainable calving pattern. It has been reported that fertility performance has declined in New Zealand dairy herds in parallel to increased milk production over the decades (Harris et al., 2006; Burke and Fowler, 2007). Though fertility performance in New Zealand dairy herds has remained stable or increased marginally in the production seasons from 2008-2009 to 2020-2021, the current national averages for key reproductive matrices are still falling short of New Zealand industry targets (LIC and DairyNZ, 2021).

Since 1990, some dairy farmers in New Zealand have adopted OAD milking (Davis et al., 1999) and in the 2015-2016 production season, about 9% of the tested herds practiced OAD milking in the entire season (Edwards, 2018b). Improved fertility performance was reported for herds milked OAD for the entire season compared with the herds milked TAD for the entire season in New Zealand (Edwards, 2018b; Hemming et al., 2018). Furthermore, breed and heterosis effects and genetic parameters for milk, fat and protein yields and fat and protein percentages have been reported in a OAD milking cow population in New Zealand (Lembeye et al., 2016a, b; Lembeye et al., 2016c). However, genetic parameters for fertility traits and the associations between milk production and fertility traits in cows milked OAD have not been estimated.

The general aim of this thesis was to estimate genetic parameters for fertility traits of spring-calved OAD and TAD milking cows in New Zealand. The specific objectives of this thesis were: 1) investigate fertility performance of spring-calved dairy cows milked in New Zealand by milking regimens and parity numbers (chapter 3); 2) estimate heritability, repeatability and individual and maternal breed, heterosis and recombination loss effects for milk production and fertility traits in cows milked OAD and TAD (chapter 4); 3) investigate phenotypic and genetic correlations between milk production and fertility traits in cows milked OAD and TAD (chapter 5); 4) identify the genes associated with milk composition and fertility traits in spring-calved dairy cows (chapter 6).

7.2 Major findings from this thesis and applications

Fertility outcomes were better for cows milked OAD for the entire lactation; they were inseminated earlier, became pregnant sooner in the mating season and calved earlier in the next season than cows milked TAD for the entire lactation. These findings are in agreement with the outcomes of previous studies for OAD milking cows in New Zealand (Clark et al., 2006; Edwards, 2018b; Hemming et al., 2018). Through the use of herd testing records, we were able to identify herds that milked OAD for part of the lactation and look at the reproductive outcomes in those herds. The key reproductive metric of PR42 did not differ between cows milked OAD for the part of the lactation and full lactation TAD. However, cows milked OAD during the mating season and cows milked OAD after peak lactation had significantly lower NIC with shorter mating length compared to cows that were milked TAD for their entire lactation. These findings will benefit farmers who are investigating a change to a whole, or part-lactation OAD milking regimen, allowing them to understand the differences in fertility performance of cows by milking regimen patterns.

Survival analysis was a more suitable method than the linear mixed model to analyse the interval fertility traits (Schneider et al., 2005). The use of survival analysis with censored time interval variables allows the inclusion of cows that did not experience the event (e.g. cows that failed to conceive) in the analysis. As described in chapter 3, proportional hazards Cox models with shared frailty of herd to account for clustering of cows within the herd is an appropriate approach to analyse censored variables. A similar approach was reported in analysing time to event fertility traits in Irish (Carty et al., 2020) and Australian (Morton et al., 2016) dairy cattle populations.

Fertility performance differed with parity number. First-parity cows had poorer fertility performance than second-, third- and fourth-parity cows, but the worst fertility performance was in cows in their fifth or greater parity. These findings help farmers to identify the cows needing extra attention prior to calving and during the mating and pregnancy period to improve their reproductive efficiency. The economic value of having old cows in both OAD and TAD milking herds and factors that are associated with poor fertility performance of cows in parities five or greater should be investigated in the future. Breed effects on milk production and fertility performance of cows milked in OAD and TAD have been reported in this thesis. In both milking populations, F cows produced greater milk yields than J cows but fertility outcomes were better with J cows than F cows. In contrast maternal breed effects for yield were in favor of J dams and maternal breed effects for fertility traits were in favor of F dams in both milking systems.

The percentage of crossbred F×J cows in the national herd continues to increase and is currently 59.2% (LIC and DairyNZ, 2022). The majority of farmers are using crossbreed cows, achieving higher production efficiency than pure breeds. Favourable individual heterosis effects were associated with milk production and fertility traits in both milking populations and were consistent across milking regimens. As discussed in chapter 4, heterosis effects for SR21, PR42, NIC and CR42 were +2.8%, +4.1%, -2.3% and +1.6%, respectively, in OAD milking cow population. Most of the estimated individual heterosis effects for fertility traits were relatively larger than the individual breed differences between F and J cows in both milking systems. Moreover, favorable maternal heterosis effects were identified in milk production traits in both milking systems, but maternal heterosis effects were less likely to influence reproductive performance (chapter 4). An absence of maternal heterosis effects for fertility traits in literature meant it was not possible to compare the findings in this thesis with the literature. Also, small heterosis effects that were found with milk fat, protein and lactose percentages are expected, because heritabilities for these traits were higher (chapters 4 and 5).

This thesis has shown a mix of favorable and unfavorable recombination loss effects for production and fertility traits in both milking systems. Most recombination effects on fertility traits were weak favourable effects with larger standard errors in TAD milking system. This is not surprising, given that recombination is expected to have a smaller impact on traits not under long-term selection, such as reproductive performance. If a separate selection scheme is implemented for OAD and TAD milking cow populations, the estimated individual breed and heterosis effects for milk production and fertility traits can be used to calculate separate breeding values and production values for milk production and fertility traits in New Zealand dairy cows.

The reported heritability and repeatability estimates for milk production and fertility traits and genetic and phenotypic correlations between yields and milk composition traits were consistent across milking regimens (chapters 4 and 5). The reported genetic parameters with milk production traits in this thesis are in agreement with previous findings by Lembeye et al. (2016a) in New Zealand. Heritabilities for milk production traits slightly greater in cows milking across all parities compared with the first parity cows in both milking populations whereas fertility traits had similar heritabilities for cows in all parities and first parity (chapters 4 and 5). The phenotypic correlations were consistent and closer to zero in both milking systems. Further research is recommended to identify the physiological factors that reduce phenotypic associations between milk production and fertility in OAD milking cow population. Results from this thesis demonstrated that most of the genetic correlations between milk production and fertility were antagonistic in both milking cow populations. The different phenotypic fertility performance observed between OAD and TAD milking systems supports the different magnitudes in genetic correlation outcomes. The antagonistic relationships between milk production and SMCO, PR42, NIC, and CR42 were weaker and closer to zero with cows milked OAD than TAD. Moreover, FSCO was negatively genetically correlated with milk and lactose yields in OAD milking cows. This reveals selection for milk production has less impact on fertility traits in OAD milking herds. Indeed, the findings in this thesis will help breeders in terms of selecting animals for OAD milking population without compromising cow fertility. Furthermore, genetic correlations between milk composition and fertility traits were positive in TAD milking population, indicating that cows producing milk with more fat, protein and lactose are more likely to be inseminated and get pregnant earlier in the mating season and calve earlier in the following calving season. This may be a consequence of selecting New Zealand dairy cattle for both high fertility and milk fat and protein percentage.

Heritability estimates for fertility traits were low (<0.1) in this thesis, which indicates that, an animal's phenotypic performance is not a good predictor of the individual's genetic merit for fertility. Thus, one of the aims of this thesis was to explore the genomic regions and genes that are associated with fertility traits of spring calved-dairy cows in New Zealand. A small genome wide association study for milk composition traits was performed as a benchmark analysis for a selected population. Highly significant associations were detected for fat and protein percentages with four SNPs on chromosome 14. Associated genes were DGAT1, SLC52A2, CPSF1 and MROH1, these genes had been identified as good candidate genes in previous GWAS in New Zealand (Ariyarathne et al., 2021; Tiplady et al., 2021). Significant associations were detected for SMFS, SMCO, FSCO, CFS, SR42, PR42, PRFS, NIC and CR42. Most of the significant SNPs for fertility traits were located on chromosome 2.

The detected candidate genes (KCNH5, HS6ST3, GLS, ENSBTAG00000051479, STAT1, STAT4, GPD2 and SH3PXD2A) for fertility traits had biological functions in metabolism, early embryonic development, fetal growth, and maternal lipid metabolism during pregnancy. Many candidate genes found to be associated with fertility traits in this thesis are novel findings and they have not been reported in previous GWAS for fertility traits in seasonal calving systems.

Overall, the reported variance components, heritability and repeatability estimates for fertility traits and genetic and phenotypic correlations of milk production and fertility traits in OAD milking cow population disclose many unrevealed aspects of genetics in cows milked OAD for the entire lactation in New Zealand dairy herds. The estimated genetic parameters in this thesis could be used to develop a selection index for fertility traits in OAD milking cow population and will be a good reference for future simulation studies. Furthermore, understanding the genetic and phenotypic relationships between milk production and fertility traits in this thesis enables the breeder to create more accurate selection indices. Since, GWAS for many fertility traits have not been published in the New Zealand dairy cattle population, the associations found between genes and key fertility measures will be a benchmark for future GWAS in New Zealand.

7.3 Limitations of the study

7.3.1 Handling of missing fertility records

Missing values for interval fertility traits existed for cows that were not assessed for the trait, and for cows that were assessed for the trait but failed to display the trait. The former are true missing values, whereas to exclude the latter would remove a biased sample of low-fertility cows. Values for cows that were assessed and failed to display the trait were replaced with a value exceeding the upper level of fertility trait to distinguish between these two groups of cows.

The genetic parameter estimation will be biased if the cows with no recorded matings or no conceptions were excluded because they were in the milking herd but did not experience the event. Therefore, missing dates were replaced with penalty values in the estimation of genetic parameters for interval fertility traits, at least partially, and in such a way keep their informative values in the analysis. In agreement with this thesis, previous studies also included the missing interval fertility traits with penalty values (Johnston and Bunter, 1996; Grosshans et al., 1997; Morton et al., 2018). In addition to that, the data augmentation technique with censored variables is described in the literature in analysing missing fertility traits (Donoghue et al., 2004; López de Maturana et al., 2007; Tiezzi et al., 2012).

7.3.2 Measuring interval fertility traits from calving to event in seasonal systems

Fertility measures from start of the mating date to the event (SMFS and SMCO) and FSCO were studied in chapter 3, excluding CFS and CI. In seasonally calving herds, the aim is to achieve as many conceptions as possible after the start of the mating date. This requires a high proportion of cows with short intervals for SMCO, SMFS and FSCO. If a cow calves late, she will have a short interval from calving to start of the mating date, and if she then conceives soon after to start of the mating date, she will also have a short CFS and CI. Thus, for this cow, her short CFS and CI will reflect the desired outcome which is a short SMCO. However, in the scenario of early calving cows, they will have a long interval from calving to start of the mating date (50-83 days), and so, even if they conceive soon after to start of the mating, they will have longer intervals for CFS and CI. When comparing CFS and CI it is be impossible to distinguish between a late-calved cow that took a long time to conceive (so had relatively unsuccessful reproductive performance) and an early-calved cow that conceived quickly. Thus, calving to event fertility measures do not accurately reflect the desirable reproductive performance of early calving cows and were not used in the comparison of reproductive performance between milking regimens.

7.3.3 Limitations in the genome-wide association study

The dairy cattle population studied for GWAS in this thesis was small to detect SNPs associated with fertility traits. In general, at least 1,000 genotyped animals are recommended in GWAS (Garrick and Fernando, 2013). The identification of strong associations and candidate genes with fat and protein percentages signifies the sample size (1537) was sufficient in chapter 6. However, associations were less likely to be detected with fertility traits in this thesis due to the low heritability (Jayawardana et al., 2023). Genetic factors with additive and non-additive effects contribute to fertility traits. Previous literature suggested that sequence-based large-sample analysis to discover most productive genomic association for complex traits in dairy cattle and associated variants is expected to grow steadily with increased sample sizes (Korte and Farlow, 2013; Daetwyler et al., 2014; Uffelmann et al., 2021). Thus, findings in this thesis require validation using a larger dataset before their implementation in genomic selection of dairy cattle fertility traits in New Zealand. In the present GWAS analysis, both genotyped and non-genotyped cows were in the pedigree, however, phenotypic traits of non-genotyped cows were not included, which is a limitation in this analysis. Further analysis is recommended, one option could be to use Bayesian methods to combine large numbers of genotyped and non-genotyped animals in the analysis (Fernando et al., 2014).

7.4 Recommendations for future research

7.4.1 Economic importance of fertility in OAD and TAD milking herds

The fertility of cows is directly related to the profitability of the farm due to its impact on farm revenues and costs. The findings of this thesis indicate that herds with cows milked OAD benefit from a higher PR42 and a lower NIC (Jayawardana et al., 2022). Thus, a higher proportion of OAD milking cows conceived to AB in the first 6-wk and fewer natural mating bulls are required. Per ‘service’ natural mating bulls are more expensive than artificial insemination, leading to reduced mating costs on OAD milking farms. Furthermore, compared with full lactation TAD milking herds, the cost of cow culling due to infertility and replacement rate and replacement cost would reduce in OAD milking herds due to better fertility performance. Also, farmers adopting OAD milking have more opportunities to remove low-production cows.

There are few studies reporting farm profitability of OAD milking compared with TAD milking systems in New Zealand (Edwards, 2018a; Edwards, 2018b). Edwards (2018a) reported; milksolids per hectare decreased by an average of 13% after adopting OAD milking and labour efficiency increased by 6%. However, the economic benefit of fertility with OAD milking has not been investigated in New Zealand. Therefore, economic modeling is recommended in future studies that accounts for the cost associated with culling, cow replacements, AB and natural mating alongside a net loss of milk production, labour requirements, milking parlor expenses, capital investments, and potentially lower cost associated with cow health in OAD milking farms. With this thesis findings, economic analysis will ultimately help farmers to make the decision of whether to use OAD milking in all or some of lactation.

At present, the fertility measure CR42 has been used to calculate the economic value of New Zealand dairy cows. The average economic value for fertility ($/CR42) estimates that a 1% change in CR42 is worth NZ$ 6.33 per cow in the year 2023 (DairyNZ, 2023). Since CR42 is greater with cows milked in OAD than TAD, the average economic value for fertility could be different between cows milking in OAD and TAD. Thus, it is worth investigating separate economic values for fertility in the OAD milking cow population.

7.4.2 Suggestions for future studies related to dairy cattle fertility traits

Based on the findings in this thesis, some aspects are recommended for future studies on the fertility of dairy cows. In this thesis, early-aged PD testing results were used to derive the conception dates of cows. However, in some cases, the last pregnancy test was recorded before the mating period end date of the herd or less than 35 days after the mating period end date of the herd. If cows were calved in the following season, it would be possible to refine these conception dates based on recorded ABs for the cow. Furthermore, cows with no 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 in the analysis. However, cow removal reasons were not compared with reproductive data sets. To be more precise it is worth exploring the missing cow records with culling or selling records in future studies. If the information on cull dates is available, these cows could be included in the survival analysis. Interval to the conception of a cow that did not conceive could be right-censored on culling date or end of mating date, which is the last day that cows could have experienced that event but the conception outcome was unknown.

In chapter 5, genetic and phenotypic correlations of only the first parity OAD and TAD milking cows for the entire lactation were investigated due to a long processing time and difficulties to get a convergence model, when data for all parities were included. Therefore, the OAD sample size was small. Further research that focuses on more OAD milking cows in consecutive production seasons would be useful. As discussed previously, genetic correlations between milk composition and fertility traits were positive in the TAD milking system, but the estimates were low in the OAD milking cow population. Many fertility traits considered in seasonal calving dairy systems occur in early lactation. Further investigation on genetic and phenotypic associations between fertility and early lactation milk composition traits (milk protein) of OAD milking cows is recommended.

Data analysis of chapter 6 was performed using the data from Massey University dairy herds. Future GWAS that focuses on milk production and fertility traits by milking regimens system with large population size would be beneficial to allow the development of genomic selection for cows in OAD milking population.

7.5 Conclusions

There are clear benefits of adoption of OAD milking for the entire lactation on dairy cattle fertility performance. Cows milked OAD for the entire lactation were more fertile than cows milked TAD for the entire lactation. Herds that transitioned to OAD milking during mating or after peak lactation had lower NIC than herds that milked TAD for the entire lactation. Fertility differed among breeds and J cows were more fertile than the F cows in both milking systems. The adoption of crossbreeding offers an opportunity for those farmers who are not already benefiting from heterosis to reap the benefits of improved milk production and fertility with minimal recombination loss in both milking populations. Importantly, the antagonistic genetic correlations between milk production and the key reproductive metrics of PR42, NIC and CR42 were less strong in cows milked OAD compared with TAD. This information is useful for cattle breeders to select animals in OAD milking environment where more emphasis can be placed on milk production with relatively less deterioration of dairy cow fertility than in TAD systems. Candidate genes that were found to be significantly associated with fertility traits in this thesis were functionally related to the reproductive performance of dairy cows in the early lactation. If associations found in this thesis are validated with larger population size, there is an opportunity to improve dairy cow fertility through genomic selection in New Zealand.

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Supplementary Materials

Genetic parameters of milk composition and fertility traits in cows milked once- or twice-daily in New Zealand

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Genetic parameters of milk composition and fertility traits in cows milked once- or twice-daily in New Zealand

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Abstract

The objective of this study was to estimate genetic and phenotypic correlations and heritabilities for milk composition and fertility traits in cows milked either once-a-day (OAD) or twice-a-day (TAD) across 21 spring-calving dairy herds in New Zealand. Fertility traits evaluated were submission in the first 3 weeks of mating (SR21), the interval from the start of mating to conception (SMCO), in-calf in the first 3 weeks of mating (PR21), and conception to the first service (PRFS). Heritability estimates of fertility traits were low and varied from 0.02 to 0.07 across the population. Phenotypic correlations between milk production and fertility traits were close to zero and most of the genetic correlations were antagonistic in both milking systems. Protein to fat ratio had positive genetic correlations with SR21, PR21 and PRFS in both milking frequencies. Estimates of genetic correlations of P:F ratio with PR21 and PRFS were relatively greater in OAD than TAD indicating that cows milked OAD had a higher P:F ratio and were more likely to get in-calf early in the breeding season than cows milked TAD.

Introduction

Genetic parameters of milk production and fertility traits have been estimated in seasonal dairy-producing systems (Grosshans et al., 1997, Berry et al., 2013). The main conclusion from cited studies is that genetic correlations between milk production and fertility traits are antagonistic. Since the late 1990s, some farmers have adopted OAD milking for the entire lactation in New Zealand (Davis et al. 1999), and for the production season of 2015-16, about 9% of the tested herds were milked OAD for the whole lactation (Edwards, 2018). Hemming et al. (2018) reported herds milked OAD for the entire lactation had better reproductive performance than those milked TAD. Genetic correlations between milk production and fertility traits have not been reported for the OAD population. Therefore, this study aimed to estimate genetic and phenotypic correlations between milk composition and fertility traits of cows milked OAD in New Zealand.

Materials and Methods

Data. Herd test milk yields, calving, mating, and pregnancy diagnosis information of spring-calved dairy cows in production season 2017-18 were extracted from the animal database of Livestock Improvement Corporation (LIC). Selected herds had at least 50 cows per herd, herd tested four or more times per lactation and at least 80% of cows in a herd had early, aged pregnancy testing information to allow conception dates to be calculated. Herds were classified as OAD or TAD for entire lactation if more than 90% of the tested cows had the same milking frequency at each herd test-day and all the herd tests were classified as the same milking regimen. After the OAD herds had been identified, the geographically closest TAD milking herd was selected within 20 km from

OAD using GPS visualizer (Schneider, 2012). The final dataset contained 4,629 milking cows from 10 OAD (2,008 cows) and 11 TAD (2,621 cows) milking herds with Holstein Friesian 816 (F), Jersey 1566 (J), and 2247 their crossbred (F×J).

Production traits. Milk production data included milk yield (MY), fat percentage (FP), protein percentage (PP), and lactose percentage (LP). Protein to fat (P:F) and protein to lactose (P:L) was calculated as the ratio between protein and fat percentages and protein and lactose percentages, respectively. Only animals with lactation lengths from 150-305 days were analysed.

Fertility traits. Four fertility traits were defined: submission for artificial insemination in the first 3 weeks of mating (SR21), interval from the start of mating to conception (SMCO), in-calf in the first 3 weeks of mating (PR21), and conception to the first service (PRFS). Pregnancy testing results where the foetal age was estimated and where cows were tested between 35-122 days (inclusive) from conception were used. Conception dates were calculated as the date of pregnancy diagnosis minus the estimated foetal age for cows with a pregnancy status of 'pregnant'. Non-pregnant cows (11.8%) were retained to estimate genetic parameters and penalized conception dates were calculated by adding 105 days (a 12-week mating period plus 21 days) to the herd start of mating date. The mating start date of each herd was sourced from the LIC database. The variables SR21, PR21 and PRFS were coded as 1 if the cow was mated or conceived in the first 3 weeks of mating, or conceived to the first service, and 0 otherwise. Cows in their first four parities were considered separately and cows in parity five and above were amalgamated into one group.

Statistical analysis. Heritability and genetic and phenotypic correlations were estimated using ASReml 4.1 (Gilmour et al., 2015). A single-trait animal model was used for the estimation of heritability for each trait and bivariate animal models were used separately to estimate the genetic and phenotypic correlations. Models included the fixed effects of herd, parity, proportion of F, heterosis, and linear and quadratic effects of deviation of calving date from the herd median calving date and the random effect of animal.

Results

Table 1: Summary statistics for milk production and fertility performance of cows milked once daily (OAD) and twice daily (TAD).

Traits[1]	OAD		TAD	
	Mean	SD	Mean	SD
MY (kg)	2696.6	686.5	4437.7	1112.0
FP (%)	5.6	0.9	5.0	0.8
PP(%)	4.1	0.3	3.9	0.3
LP(%)	4.9	0.2	5.0	0.2
SMCO (days)	21.1	17.8	24.6	19.4
SR21 (%)	85.6	35.2	83.1	37.5
PR21 (%)	58.6	49.3	48.7	50.0
PRFS (%)	68.3	46.5	60.9	48.8

[1] MY= milk yield; FP= fat percentage; PP=protein percentage; LP= lactose percentage; SMCO=start of mating to conception; SR21= proportion of cows that presented for mating within 21 days of the start of mating; PR21 = proportion of cows that conceived within 21 days of the start of mating; PRFS = proportion of cows that conceived to their first service.

Cows milked TAD produced greater MY and higher LP than those cows milked in OAD (Table 1), but had poorer fertility outcomes. Fat and protein percentages were higher in cows milked OAD (Table 1). Compared with cows milked TAD, a higher proportion of cows milked OAD were mated (>2.5%) and conceived (>9.9%) within 3 weeks of the mating season and were pregnant (>7.4%) to their first AB.

Table 2: Heritabilities, genetic and phenotypic correlations of milk production and fertility traits for cows milked in once daily (OAD) and twice daily (TAD).

	Traits[1]	MY	FP	PP	LP	P:F	P:L	SMCO	SR21	PR21	PRFS
OAD	MY	**0.36**	-0.30	-0.27	0.11	0.13	-0.28	-0.02	0.01	-0.04	0.03
	FP	-0.53	**0.64**	0.54	-0.11	-0.79	0.50	-0.05	0.03	0.03	0.01
	PP	-0.65	0.64	**0.72**	-0.12	0.03	0.89	-0.12	0.09	0.11	0.07
	LP	0.01	0.01	-0.13	**0.40**	0.01	-0.56	-0.04	0.01	0.01	0.04
	P:F	0.11	-0.77	-0.04	-0.11	**0.67**	0.03	-0.02	0.02	0.03	0.04
	P:L	-0.57	0.55	0.93	-0.49	0.01	**0.65**	-0.08	0.07	0.08	0.03
	SMCO	-0.03	0.18	-0.36	0.62	-0.45	-0.49	**0.04**	-0.25	-0.74	-0.55
	SR21	0.06	-0.51	-0.29	-0.19	0.46	-0.13	-0.36	**0.02**	0.38	0.21
	PR21	-0.39	-0.20	0.16	-0.59	0.38	0.33	-0.69	0.44	**0.05**	0.54
	PRFS	-0.16	-0.28	0.09	-0.44	0.44	0.22	-0.97	0.60	0.97	**0.04**
TAD	MY	**0.30**	-0.30	-0.29	0.02	0.18	-0.24	0.01	0.02	-0.03	0.01
	FP	-0.72	**0.65**	0.61	-0.02	-0.82	0.48	-0.03	0.03	0.04	0.01
	PP	-0.63	0.61	**0.67**	-0.05	-0.09	0.81	-0.09	0.07	0.08	0.05
	LP	-0.14	0.10	0.25	**0.21**	-0.03	-0.59	0.01	0.03	0.01	0.03
	P:F	0.59	-0.85	-0.33	-0.03	**0.40**	-0.05	-0.02	0.02	0.01	0.02
	P:L	-0.59	0.78	0.98	-0.36	-0.34	**0.29**	-0.07	0.04	0.06	0.02
	SMCO	0.24	-0.05	-0.30	-0.04	-0.23	-0.28	**0.07**	-0.24	-0.72	-0.58
	SR21	-0.04	-0.34	0.11	-0.27	0.70	0.29	-0.98	**0.04**	0.33	0.11
	PR21	-0.48	0.08	0.33	0.38	0.25	0.16	-0.80	0.96	**0.05**	0.59
	PRFS	-0.46	0.15	0.38	0.57	0.10	0.06	-0.96	0.29	0.99	**0.02**

Genetic correlations are shown below the diagonal, phenotypic above, and heritabilities along in the diagonal. Standard errors for all phenotypic and genetic correlations were ≤ 0.03 and ≤ 0.53 in both milking frequencies respectively. [1]MY= milk yield; FP= fat percentage; PP=protein percentage; LP= lactose percentage; P:F= protein to fat ratio; P:L= protein to lactose ratio; SMCO=start of mating to conception (days); SR21= proportion of cows that presented for mating within 21 days of the start of mating; PR21 = proportion of cows that conceived within 21 days of the start of mating; PRFS = proportion of cows that conceived to their first service.

Heritability of production and fertility traits varied from 0.22 to 0.72 and 0.02 to 0.05 in OAD, from 0.13 to 0.67 and 0.02 to 0.07 in TAD respectively (Table 2). In both milking systems, the genetic correlations of MY with FP, PP, and P:L were moderate and negative. Milk yield was moderately positively correlated with P:F in TAD. In both milking systems there were strong negative genetic and phenotypic correlations between FP and P:F, while correlations of FP with PP and P:L were positive and moderate to strong. Genetic and phenotypic correlations between PP and P:L were strongly positive but LP and P:L were moderately negatively correlated in both OAD and TAD herds.

In general, phenotypic correlations between milk production and fertility traits were low and close to zero for both milking frequencies. Most genetic correlations between milk production and fertility traits were negative and low to moderate. Genetic correlations of MY with PR21 and PRFS were moderate and negative in TAD, which was relatively greater compared to OAD. In OAD milking, FP was moderate and negatively correlated with SR21, PR21 and PRFS. In both milking frequencies, genetic correlations of PP, P:F and P:L with PR21 and PRFS were positive and low to moderate whereas PP, P:F and P:L were moderate negatively correlated with SMCO. Genetic correlations with P:F and SR21 were positive in OAD and TAD, but relatively greater in TAD. Genetic correlations of LP with SMCO were positive and moderate in OAD, but close to zero in TAD. Genetic correlations of LP with PR21 and PRFS were positive and moderate in TAD, but this was moderately negatively correlated in OAD.

Discussion

Heritability estimates and phenotypic and genetic correlations between milk yield and compositional traits in cows milked OAD and TAD for this study were consistent with Lembeye et al. (2016) in New Zealand. Estimated heritabilities for fertility traits were low and similar to previous findings (Grosshans et al., 1997; Berry et al., 2013). The low heritability of fertility traits are a consequence of small genetic variation and relatively large residual variation of cows in both milking frequencies. Our results indicate that increases in MY were associated with a decrease in submission and in-calf and lengthening SMCO. This antagonistic genetic relationship between production and fertility of cows implies that selecting only for cows with higher milk production leads to a genetic decline in cow fertility. For this reason, New Zealand and most other national genetic evaluation systems now include fertility in their selection indices.

Our findings show that cows with a higher PP, P:F and P:L ratio, are more likely to get pregnant early in the breeding season and to conceive to their first service in both milking populations. A positive genetic association of PP with fertility has been reported by Morton et al. (2018), highlighting better fertility in cows with higher milk PP. Though the genetic correlations of PP with PR21 and PRFS are higher in TAD compared to OAD milking, genetic correlations of P:F and P:L with fertility are comparatively greater in OAD than TAD milking cows. This indicates that herds milked OAD have better fertility and a higher milk solids percentage, so each kilogram of milk provides a greater return to the farmer. The reason for the negative genetic correlation between FP and fertility outcomes with OAD milking in this study has not yet been identified. Davis et al. (1999) reported that LP decreased in OAD milking cows due to changes in mammary permeability which enhanced efflux of lactose through mammary tight junctions. The negative association between LP and fertility in cows milked OAD should be further investigated. The findings of this study indicate that there is potential to increase the fertility performance of dairy cattle by identifying the causes of the association between milk protein concentration and fertility of cows.

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Milk production and fertility of spring-calved Holstein-Friesian, Jersey and crossbred cows milked once daily or twice daily in New Zealand

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MILK PRODUCTION AND FERTILITY OF SPRING-CALVED HOLSTEIN-FRIESIAN, JERSEY AND CROSSBRED COWS MILKED ONCE DAILY OR TWICE DAILY IN NEW ZEALAND

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SUMMARY

In New Zealand about 55% of dairy herds are milked twice daily (TAD) and about 9% of herds are milked once daily (OAD) for their entire lactation, with the balance of herds using variable milking frequencies across lactation. The objective of this study was to investigate fertility of spring-calved Holstein-Friesian (F), Jersey (J) and crossbred of Holstein-Friesian × Jersey cows (F×J) milked either OAD or TAD from 2015-2016 to 2017-2018 in New Zealand using data provided by Livestock Improvement Corporation. The dataset comprised 113 OAD and 531 TAD herds. Eight fertility traits were evaluated: submission in the first 3 weeks (SR21) and 6 weeks (SR42) of mating, in-calf in the first 3 weeks (PR21) and 6 weeks (PR42) of mating, conception to first service (PRFS), not in-calf at end of the breeding season (NIC), 3-week calving (CR21) and 6-week calving (CR42) rates. Cows milked TAD produced greater milk, fat, protein and lactose yields than cows milked OAD, but fat (FP) and protein percentages (PP) were lower in cows milked TAD. Cows milked OAD had better fertility with a higher SR21, PR21, PR42, PRFS, CR21, CR42 and a lower NIC than cows that were milked TAD. Breeds differed in fertility traits in both milking regimens. Jersey and F×J cows had higher SR21, SR42 and PRFS than F cows in OAD milking herds, whereas J cows were mated earlier in the mating season than F and F×J cows in TAD. Fertility of F×J cows was better than purebred cows in both milking populations, evidenced by these cows having the highest PR21, PR42, PRFS, CR21, CR42 and lowest NIC. Once daily milking herds benefited from higher FP and PP and better fertility than TAD herds.

INTRODUCTION

Once-daily milking is becoming popular among New Zealand dairy farmers because it benefits the farmers lifestyle, animal welfare, management of feed shortfalls and reduces the cost of labour (Bewsell *et al.*, 2008). In the production year 2015-2016, about 9% of herd-tested herds milked OAD for the whole lactation in New Zealand (Edwards, 2018). Improved reproductive performance was reported with spring-calved cows milked OAD for the entire lactation compared to cows milked TAD for the entire lactation in New Zealand (Jayawardana *et al.*, 2022).

The New Zealand dairy herd is comprised of crossbreed of F×J cows (49.6%), F (32.5%), J (8.2%), other breeds and crosses (9.3%) and a small proportion of Ayrshire (0.4%), (LIC and DairyNZ, 2021). Grosshans *et al.* (1997) reported breed differences in reproductive performance of New Zealand dairy cows, including shorter intervals from calving and mating to first service and conception, and higher 6-wk in-calf rates in J cows compared to their F counterparts. Lembeye *et al.* (2016) reported J cows milked OAD for their entire lactation were more efficient per kg of live weight than F and F×J cows milked OAD, but suggested that F×J cows are more suitable for OAD milking due to greater total milk solids production, intermediate feed conversion efficiency and biological efficiency. The information of breed differences in reproductive performance in OAD milking system is scare at cow level. The objective of present study was to evaluate the reproductive performance of F, J and crossbred F×J cows milked either OAD or TAD in New Zealand using data from a national herd-testing database.

MATERIALS AND METHODS

Data. Herd test milk yields, calving, mating, and pregnancy diagnosis information of spring-calved dairy cows from 2015-2016 to 2017-2018 production seasons were obtained from the animal database of Livestock Improvement Corporation. Selected herds had at least 50 cows per herd, herd tested four or more times per lactation, pregnancy test results recorded for at least 80% of cows that calved in the 12-month period, and "early aged pregnancy testing" (tested on or between 35 and 122 days of pregnancy) and fetal age estimated for at least 80% of cows in the herd. Herd test-day milking frequency was used to classify herds into OAD or TAD. If more than 90% of the tested cows on a herd-test date were milked either OAD or TAD in a herd, then it was classified as OAD or TAD milking herd on that herd-test date. Likewise, all herd tests were classified. If all herd tests were OAD throughout the season, then the herd was identified as an OAD milking herd. Likewise, if all herd tests were classified as TAD, then the herd was identified as a TAD herd. Finally, 113 OAD and 531 TAD herds were identified. Herds that were OAD at some herd tests and TAD at other herd tests were excluded.

Breeds. Information of breed composition (expressed in sixteenths) for each cow was used to classify the cows into 3 breed categories; F, J, and crossbred of F×J. Herds without F, J or crossbred of F×J cows were excluded. Cows with either less than 100% known breed proportions or more than 12.5% of any breed other than F or J were excluded. Cows were classified as F or J if they had breed compositions of F ≥14/16 or J ≥14/16, respectively and remaining cows were classified as crossbred of F×J.

Production traits. Milk production data included yields of (MY), fat (FY), protein (PY), lactose (LY) and percentages of fat (FP), protein (PP), and lactose (LP). Lactation records with days in milk ranging 150-305 days were analysed.

Fertility traits. Eight fertility traits were defined: submission for artificial insemination in the first 3 weeks (SR21) and 6 weeks (SR42) of the breeding season, in-calf in the first 3 weeks (PR21) and 6 weeks (PR42) of the breeding season, conception to the first service (PRFS), not in-calf at end of the breeding season (NIC), calving by first 3 weeks (CR21) and 6 weeks (CR42) from the planned start of the calving. Conception dates were calculated as the date of pregnancy diagnosis minus the estimated foetal ages with a pregnancy status of ‘pregnant’. If the estimated foetal ages were not available but cows calved in the following season, their conception date was calculated as calving date in the following season minus 282 days. Submission by 3 weeks or 6 weeks of the breeding season was coded as 1 if the first mating date was in the first 21 days or 42 days from the start of mating date, respectively, otherwise coded as 0. Likewise, in-calf by 3 weeks or 6 weeks of the breeding season was coded as 1 if the cow was pregnant in the first 21 days or 42 days from the start of breeding season, respectively, otherwise coded as 0. The variable PRFS was only calculated for cows whose first service was to artificial breeding, and was coded as 1 for cows where date of first service equalled date of conception, and 0 otherwise. Pregnancy status at the last pregnancy testing after the end of the breeding period was used to classify the NIC, cows with pregnancy status ‘empty’ were coded as 1 whereas cows with pregnancy status ‘pregnant’ were coded as 0. Cows with last pregnancy status as doubtful but calved in the subsequent season were coded as 0, otherwise 1. Planned start of calving date was obtained for a herd by adding 282 d to the herd’s mating start date in each calving season. If a cow calved in the first 3 weeks or 6 weeks from the planned start of calving date then it was coded as 1, otherwise 0. The detailed description of editing the fertility traits and calculation of conception in the present study was described in Jayawardana *et al.* (2023). Cows in their first four parities were considered separately and cows of parity five and above were combined into one category.

Statistical analysis. The statistical analyses were undertaken using SAS version 9.4 software. The production traits were analysed using HPMIXED procedure and all fertility traits with binomial distribution were analysed using the GLIMMIX procedure after a logit transformation. Contemporary groups were defined as the group of cows calving in the same herd and year. The model included the fixed effects of milking regimen, breed, parity, interaction of milking regimen and breed, linear and quadratic effects of deviation of calving date from the herd median calving date (within-herd in each calving year) as covariates, and the random effects of herd-year and residual. Least-squares means with logit scale were back-transformed into the nominal scale for interpretation of the results.

RESULTS AND DISCUSSION

Cows milked TAD produced greater yields of milk, fat, protein and lactose and higher LP than cows milked OAD, but had lower FP and PP and poor fertility outcomes. Results indicate that a higher proportion of cows milked OAD were mated (by 4.6%) in the first 3 weeks of the breeding season, conceived in the first 3 weeks (by 10%) and 6 weeks (by 8.6%) of the breeding season, pregnant to their first service (by 6.8%), calved by 3 weeks (by 6.2%) and 6 weeks (by 4.6%) of the following calving season with a lower percentage not in-calf (by 3.7%) at end of the breeding season compared with TAD milking cows. The better reproductive performance of OAD milking cows is hypothesised to be due to OAD milking reducing the extent of negative energy balance in the early lactation cows (Kay *et al.*, 2013).

Table 1: Least-squares means of milk production and fertility traits of Holstein-Friesian (F), Jersey (J) and their crossbred cows (F×J) milked in once daily (OAD) or twice daily (TAD)

Traits[1]	OAD	TAD	OAD			TAD			P-value		
			F	J	F×J	F	J	F×J	MF	Breed	MF × Breed
MY(kg)	3291	4708	3595^{a}	2936^{c}	3368^{b}	5115^{d}	4102^{f}	4828^{e}	<.001	<.001	<.001
FY(kg)	170.4	228.9	171.1^{b}	165.2^{c}	175.9^{a}	230.1^{e}	220.4^{f}	235.2^{d}	<.001	<.001	<.001
PY(kg)	134.6	182.1	141.2^{a}	125.3^{c}	138.8^{b}	190.6^{d}	166.7^{f}	187.1^{e}	<.001	<.001	<.001
LY(kg)	162.1	236.4	176.4^{a}	145.5^{c}	166.4^{b}	255.6^{d}	207.1^{f}	242.6^{e}	<.001	<.001	<.001
FP(%)	5.29	4.98	4.82^{c}	5.68^{a}	5.28^{b}	4.55^{f}	5.51^{d}	4.93^{e}	<.001	<.001	<.001
PP(%)	4.13	3.91	3.95^{c}	4.29^{a}	4.14^{b}	3.75^{f}	4.10^{d}	3.89^{e}	<.001	<.001	<.001
LP(%)	4.94	5.03	4.90^{c}	4.96^{a}	4.94^{b}	5.00^{f}	5.06^{d}	5.04^{e}	<.001	<.001	<.001
SR21(%)	85.3	80.7	83.9^{b}	86.6^{a}	86.8^{a}	78.5^{e}	82.5^{c}	81.2^{d}	<.001	<.001	0.30
SR42(%)	93.9	93.7	92.4^{b}	94.8^{a}	94.7^{a}	92.4^{e}	94.9^{c}	94.0^{d}	0.65	<.001	0.11
PR21(%)	55.4	45.4	53.7^{b}	55.7^{b}	57.8^{a}	43.8^{e}	45.1^{d}	47.1^{c}	<.001	<.001	0.94
PR42(%)	76.5	67.9	74.1^{c}	77.1^{b}	78.9^{a}	65.9^{f}	67.8^{e}	69.7^{d}	<.001	<.001	0.28
PRFS(%)	62.1	55.3	60.0^{b}	62.9^{a}	63.5^{a}	54.4^{d}	54.5^{d}	56.6^{c}	<.001	<.001	0.06
NIC(%)	9.8	13.5	10.5^{a}	9.5^{a}	8.6^{b}	14.8^{c}	13.4^{d}	12.4^{e}	<.001	<.001	0.92
CR21(%)	64.2	58.0	64.0^{a}	64.1^{a}	65.8^{a}	57.0^{c}	57.0^{c}	59.6^{b}	<.001	<.001	0.69
CR42(%)	86.6	82.0	85.7^{b}	86.5^{b}	87.9^{a}	81.0^{d}	82.2^{c}	82.9^{c}	<.001	<.001	0.35

[a-f] Means with different superscripts in the same row are significantly different across milking regimen and breeds ($P < 0.05$).

[1] MY = milk yield; FY = fat yield; PY = protein yield; LY = lactose yield; FP = fat percentage; PP = protein percentage; LP = lactose percentage; SR21 = cow inseminated in the first 3 weeks from the start of mating; SR42 = cow inseminated in the first 6 weeks from the start of mating; PR21 = cow conceived in the first 3 weeks from the start of mating; PR42 = cow conceived in the first 6 weeks 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 weeks from the planned start of the calving; CR42 = cow calved in the first 6 weeks from the planned start of the calving; MF= milking frequency.

In both milking systems, F cows had greater MY, PY and LY compared with J and F×J cows, but FY was higher for crossbred F×J cows than purebred F and J cows. Jersey cows were less affected than F and F×J cows by OAD milking with a reduction in MY, PY and FY ranged 25-28%, whereas in F and F×J cows the reduction ranged between 26% to 30%. Fat, protein and lactose percentages were higher in J cows and lowest in F cows in both milking populations. Sneddon *et al.* (2015) reported that J milk was most valuable per litre in New Zealand under the milk product portfolios of whole-milk powder, skim-milk powder, cheese and butter. Milk from J milked OAD has the highest value per litre, due to the increase in fat and protein percentage. Significant interactions were found between milking frequency and breed for milk production traits in this study. However, no milking frequency × breed interactions were detected for any fertility traits. Similarly, in the experimental study by Clark *et al.* (2006), J and crossbred F×J cows were submitted for mating at similar rates, but F×J cows had superior PR42 and NIC rates than J cows. This suggests that conception rates were higher in F×J cows than J cows. Jayawardana *et al.* (2023) reported that heterosis effects of F×J cows for SR21 was lower than PR21 and PR42 in OAD (SR21=2.8% vs PR21=5.5% and PR42=4.1%) and TAD (SR21=3.1% vs PR21 and PR42=5.8%) milking systems. Across both milking regimens crossbred F×J cows had the best overall reproductive performance, and F cows had the worst reproductive performance.

CONCLUSIONS

Cows milked OAD for the entire lactation had higher FP, PP and better fertility outcomes than cows milked TAD during the entire lactation. Fertility differed among breeds in both milking systems. Jersey cows were presented earlier for mating than F cows. Crossbred F×J cows had better fertility than purebred F and J cows, they became pregnant sooner in the mating season, and calved earlier in the following season than F and J cows in both milking populations.

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Name/title of Primary Supervisor:	Prof. Nicolas Lopez-Villalobos

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