Associations between postpartum phenotypes, cow factors, genetic traits, and reproductive performance in seasonal-calving, pasture-based lactating dairy cows

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ABSTRACT

The objective of this study was to evaluate associations between corpus luteum (CL) status, uterine health, body condition score (BCS), metabolic status, parity, genetic merit for fertility traits, and reproductive performance in pasture-based dairy cows managed for seasonal reproduction. First- and second-lactation (n = 2,600) spring-calving dairy cows from 35 dairy farms located in Ireland were enrolled in the study. Farms were visited every 2 wk, and animals that were at wk 3 (range: 14–27 d in milk) and wk 7 (range: 42–55 d in milk) postpartum were examined. Body condition score was measured using a 1-to-5 scale in 0.25-point increments. Transrectal ultrasound examination was performed at wk 3 and 7 postpartum to determine presence or absence of CL and ultrasound reproductive tract score (scale of G1–G4). Blood samples were collected at each visit, and the concentrations of glucose, β-hydroxybutyrate (BHB), and fatty acids (FA) were analyzed using enzymatic colorimetry. Animals were grouped into 3 BCS categories [low (≤2.5), target (2.75–3.25), and high (≥3.5)], 2 CL categories (present or absent), 2 uterine health status categories (normal or abnormal), and 3 metabolic status categories [good (high glucose, low FA and BHB), poor (low glucose, high FA and BHB), and moderate (all other combinations)]. Fisher's exact test was used to test for associations between variables and was supplemented by logistic regression. More cows with a CL at wk 7 were served during the first 21 d of the breeding period compared with cows without a CL. Cows classified as having a uterine score of G3 or G4 at wk 3 and 7 had lower odds of pregnancy establishment during the breeding period compared with animals with a uterine score of G1 or G2. Animals with low BCS at wk 7 had lower odds of pregnancy establishment than cows with a target BCS. Cows classified as having good metabolic status at both wk 3 and wk 7 had greater odds of pregnancy establishment during the first 21 d of the breeding season than those classified as having poor metabolic status. Overall, primiparous cows had greater reproductive performance than second-parity cows. Animals in the quartiles with the best predicted transmitting ability for survival and calving interval had better reproductive performance compared with animals in the other quartiles. Cows that had better genetic merit for fertility traits and good metabolic status, achieved target BCS, and had a favorable ultrasound reproductive tract score and a CL present at wk 7 postpartum had superior reproductive performance.
Key words: uterine infection, anestrus, fertility, genetic merit for fertility traits

INTRODUCTION

Pasture-based dairy systems managed for seasonal calving require a 12-mo calving interval to maximize pasture nutrient utilization (Dillon et al., 2006). Excellent reproductive performance in this production system is necessary to obtain a concentrated calving pattern during late winter and early spring (Morton, 2010), and calving pattern is a key driver of farm profitability (Shalloo et al., 2014).

It has been reported that approximately 11 to 38% of cows are anovular at the beginning of the breeding season, and this can be a factor that limits herd submission rates (Rhodes et al., 2003). The beneficial effect of early resumption of ovarian cyclicity remains controversial. Some studies reported that early ovulation resulted in greater pregnancies per AI (**P/AI**) and

shorter calving to conception interval (Galvão et al., 2010; Bittar et al., 2014); others, however, reported delayed uterine involution (Heppelmann et al., 2013) and detrimental effects on reproductive performance (Smith and Wallace, 1998).

Sheldon et al. (2006) proposed a case definition for clinical endometritis as the presence of purulent discharge detectable in the vagina at 21 d or more after parturition. Absence of purulent vaginal discharge does not exclude endometritis (Šavc et al., 2016), and it might lead to an erroneous diagnosis of endometritis instead of vaginitis. Ultrasound examination may help to identify pathological conditions in the uterus (Quintela et al., 2012). The amount of fluid and uterine lumen shape detected by ultrasound have been reported to be correlated with the existence of bacterial growth in uterine fluid samples (Mateus et al., 2002) and reproductive performance (Mee et al., 2009; Jaureguiberry et al., 2017).

Temporal changes in BCS are a key driver of cow health and fertility (Berry et al., 2008); BCS at calving, nadir BCS, and BCS losses during the postcalving period have been associated with reproductive performance in confinement (López-Gatius et al., 2003) and pasture-based systems (Roche et al., 2007). Ospina et al. (2010b) reported that animals that had postpartum blood concentrations of fatty acids (**FA**) ≥ 0.72 mEq/L or BHB ≥ 0.96 mmol/L had a decreased risk of pregnancy within 70 d after the voluntary waiting period. Conversely, greater circulating concentrations of glucose during the prepartum period and the first 3 wk postpartum have been associated with shorter intervals to pregnancy (Cardoso et al., 2013) and greater odds of pregnancy at first service (Garverick et al., 2013).

Genetic merit for fertility traits affects several organs and tissues involved in fertility performance in dairy cows (Butler, 2013). Animals with poor genetic merit for fertility traits have lesser BCS, unfavorable metabolic status, longer postpartum anestrous intervals, increased incidence of endometritis, inflammation, dysfunctional estrous behavior, and lesser plasma progesterone concentrations during the luteal phase (Cummins et al., 2012b; Moore et al., 2014a; Moran et al., 2017). However, no studies have assessed the associations between individual genetic traits and reproductive performance in dairy cows managed under a seasonally calving, pasture-based system. Therefore, the aim of this study was to identify factors associated with postpartum fertility phenotypes in pasture-based, seasonal-calving dairy cows. Specifically, the objective was to develop a large data set of first- and second-parity cow records to evaluate the associations between corpus luteum (**CL**) presence, uterine health, BCS, metabolic status, parity, individual genetic traits, and reproductive performance in seasonal-calving, pasture-based lactating dairy cows.

MATERIALS AND METHODS

All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee and authorized by the Health Products Regulatory Authority, which is the competent authority in Ireland responsible for the implementation of European Union legislation (Directive 2010/63/EU) for the protection of animals used for scientific purposes.

Herds and Experimental Design

A prospective, observational cross-sectional study was conducted on 35 pasture-based, seasonal-calving dairy herds located in the province of Munster in Ireland. Within each herd, the study population included first- (n = 1,637) and second- (n = 1,074) lactation dairy cows. All enrolled cows calved during the spring season (February to April) in 2015 (n = 24 herds) and 2016 (n = 11 herds). Only cows that were ≥ 30 DIM on the planned farm mating start date (**MSD**) were enrolled. Cows were excluded or withdrawn from the study if the herd owners observed clinical diseases, which included mastitis, lameness, metabolic disorders, and displaced abomasum.

Animals were divided into 3 calving categories based on DIM at the farm MSD. Early-calving cows (n = 1,331) were ≥ 70 DIM at MSD, mid-calving cows (n = 778) were 42 to 69 DIM at MSD, and late-calving cows (n = 486) were 30 to 41 DIM at MSD.

The study data collection schedule is outlined in Figure 1. Examinations were performed at wk 3 (14–27 DIM) and wk 7 (42–55 DIM) postpartum, between 7 to 14 d after first service during the breeding period and 5 to 7 wk after the farm mating end date. To collect all the data at the desired time points, researchers visited all enrolled herds every 2 wk during the postpartum period and every week during the breeding period. The postbreeding visit was carried out once between 34 and 50 d after the farm mating end date to determine pregnancy status and estimate fetal age. At the wk 3 and 7 visits, postpartum ovarian structures and uterine health were evaluated by transrectal ultrasound (8.5-MHz transrectal transducer; Ibex Pro, E.I. Medical Imaging, Loveland, CO). Body condition score was evaluated by a single observer using a 1-to-5 scale (1 = emaciated; 5 = obese) with 0.25-point increments (Edmonson et al., 1989). Cows were classified according to their BCS as low (BCS ≤ 2.5), target (BCS 2.75–3.25), or high (BCS ≥ 3.5).

Ultrasound Evaluation of Ovarian Status and Reproductive Tract Score

The presence or absence of a CL was recorded during the ultrasound evaluation. Cows with either a follicular cyst (>25 mm diameter and no CL) or a luteal cyst were noted but removed from the analysis. Ultrasound reproductive tract score (**URTS**) was determined by examining the uterine horns based on criteria outlined by Mee et al. (2009) as follows: G1 = a typical spoke wheel–shaped lumen was observed; G2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity was observed; G3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity was observed; G4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity was observed. These URTS were used to create a binary variable describing uterine health status (**UHS**). The UHS variable was created due to a low number of animals in the G1 (n = 54) and G4 (n = 22) uterine categories at wk 3 and 7, respectively, which was consistent with expected physiological postpartum uterine involution. At wk 3, cows diagnosed as having a uterine score of G1 or G2 were considered to have normal UHS, and cows diagnosed as having a uterine score of G3 or G4 were considered to have abnormal UHS. At wk 7, cows diagnosed as having a uterine score of G1 were considered to have normal UHS, and cows diagnosed as having a uterine score of G2, G3, or G4 were considered to have abnormal UHS. Most cows exhibited improvement in URTS (and hence UHS) between wk 3 and 7; therefore, we did not calculate a UHS change variable for use in the data analysis.

Blood Sampling and Metabolic Status Assays

Blood samples were collected via coccygeal venipuncture into evacuated lithium heparin tubes (Vacutainer, Becton Dickinson, Meyla, UK) and stored at 4°C during transportation. Samples were centrifuged for 15 min at 1,500 × g, and plasma was harvested and stored at −20°C until further analysis. The plasma concentrations of glucose, BHB, and FA were analyzed using enzymatic colorimetry (ABX Mira, Montpelier, France). The glucose kits were supplied by Horiba ABX (Montpellier, France), the BHB kits were supplied by Randox Laboratories Ltd. (Crumlin, UK), and the FA kits were supplied by Wako Chemicals GmBH (Neuss, Germany). Cows were categorized as having low glucose, high FA, and high BHB when plasma concentrations met the following criteria: glucose <60.0 mg/dL, FA >0.60 mEq/L, and BHB >0.76 mmol/L (Ospina et al., 2010a; Garverick et al., 2013).
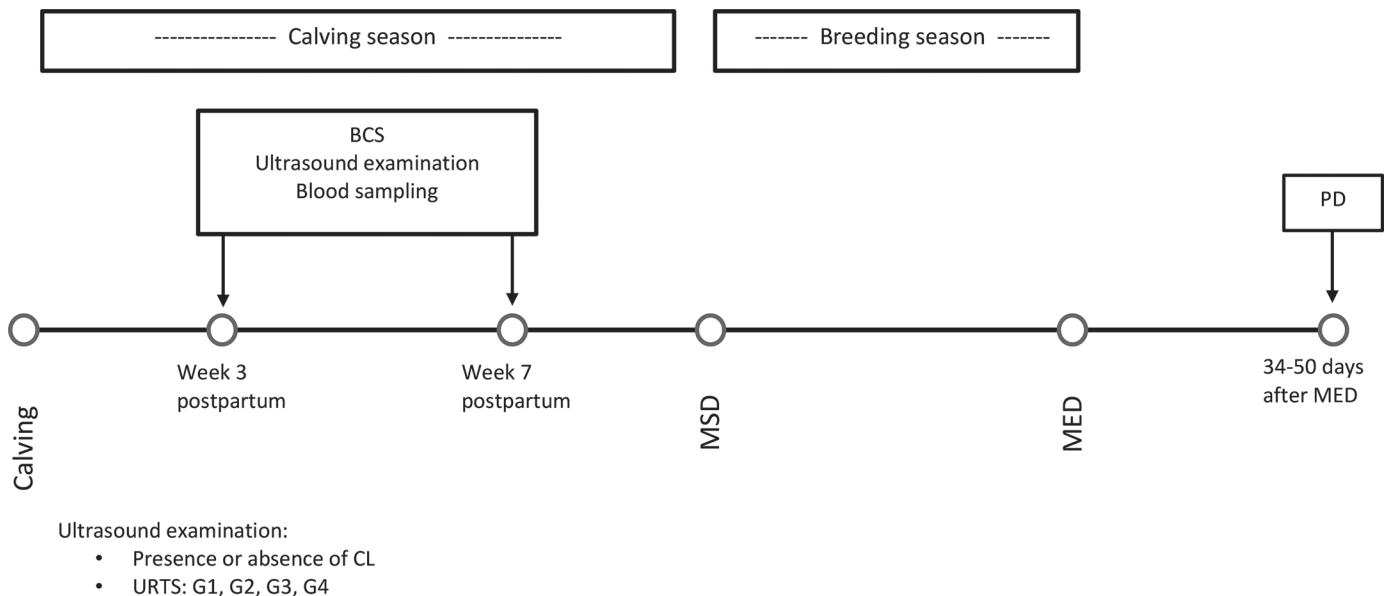

Figure 1. Diagram of activities. During the calving and breeding seasons, farms were visited every 2 wk and every week, respectively. MSD = mating start day; MED = end of breeding season; PD = pregnancy diagnosis by ultrasound; CL = corpus luteum. URTS = ultrasound reproductive tract score [G1 = a typical spoke wheel–shaped lumen; G2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity; G3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity; G4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity].

Reproductive Parameters

Reproductive records including farm MSD, service dates, and the mating end date were obtained from the Irish Cattle Breeding Federation profile for each participating herd. The 21-d submission rate (**SR21**) was constructed by coding cows with an insemination date within the first 21 d after MSD as 1 and coding those with no insemination date within the first 21 d as 0. Pregnancy to first insemination (**P/AI1**) was coded as 1 if an animal received only 1 service and was diagnosed as pregnant at the end of the breeding season. Cows with more than 1 service or that were diagnosed as nonpregnant were allocated a P/AI1 of 0. Pregnancy to second insemination (**P/AI2**) was coded as 1 if an animal received 2 services and was diagnosed as pregnant at the end of the breeding season. Cows with more than 2 services or that were diagnosed as nonpregnant were allocated a P/AI2 of 0.

Pregnant within 21 d of the onset of breeding (**P21**) was coded as 1 if cows had at least 1 service during the first 21 d of the breeding period and did not receive another service after 21 d of the breeding period and were subsequently confirmed as pregnant. A cow received a P21 record of 0 if a service was obtained sometime after 21 d of breeding or if the animal was diagnosed as nonpregnant. Similar descriptions were used for pregnant within 42 d (**P42**) and 84 d (**P84**) after MSD. The MSD to conception interval (**MSD-CI**) was calculated as the interval in days from MSD until the service event that resulted in pregnancy establishment.

Data Handling and Statistical Analysis

The independent variables investigated were CL status, URTS, UHS, BCS, BCS change, metabolic status, calving date, and parity. For each variable, a cohort of cows was designated as the reference group for determination of odds ratios (**OR**; = 1). An OR of >1 implies increased likelihood and an OR of <1 implies decreased likelihood of a particular outcome relative to the reference group. Cows were assigned to 1 of 4 BCS change categories (change in BCS between wk 3 and 7 postpartum) as follows: lost ≥0.5, lost 0.25, kept constant, and gained ≥0.25. Blood indicators of metabolic status were assigned to 1 of 3 categories: good (high glucose, low FA and BHB), poor (low glucose, high FA and BHB), and moderate (all other possible combinations). On wk 7, due to the low number of animals in the poor category (n = 66), only 2 groups were included in the metabolic status analysis: good and moderate–poor combined. The binary dependent variables investigated were SR21, P/AI1, P/AI2, P21, P42, and P84. To evaluate the marginal associations between independent variables and binary and multinomial dependent variables, Fisher's exact test was used and was supplemented by multiple logistic regression using the LOGISTIC procedure of SAS (version 9.3; SAS Institute Inc., Cary, NC) to calculate OR and predicted probabilities, including herd, year, parity, and calving date as fixed effects. The postpartum phenotype variables measured at multiple time points (wk 3, wk 7, at AI) and variables where the change between wk 3 and wk 7 was calculated were analyzed individually. The GLIMMIX procedure was used to determine the association between independent variables and MSD-CI, including herd, year, parity, and calving date as fixed effects.

Each subindex of the economic breeding index comprises individual genetic traits (ICBF, 2017). For this study, the fertility subindex and specific individual genetic traits [PTA for calving interval, survival, milk (kg), and milk protein (%)] were selected for inclusion in the analysis. Calving interval and survival are defined as number of days between successive calving events and longevity in the herd, respectively. Initially, separate logistic regression models were developed for each index as a continuous variable. Then, each genetic trait and the fertility subindex were stratified into quartiles, and logistic regression models were used to calculate OR and predicted probabilities, including herd, year, parity, and calving date as fixed effects even if they were nonsignificant.

The effects of independent variables on the MSD-CI were evaluated using the Kaplan–Meier survival analysis test (the LIFETEST procedure). Cows that were still not pregnant at the end of the breeding period were right-censored at 84 d (i.e., all cows were allowed a maximum potential breeding period of 12 wk).

RESULTS

The postpartum CL status, URTS, UHS, metabolic status, and BCS phenotypes, and their associations with each other and with parity and genetic traits were described by Rojas Canadas et al. (2020). The current paper describes the relationship between these variables and reproductive performance. The overall mean reproductive performance outcomes across all enrolled herds were SR21 = 93.7% (2,573/2,745), P/AI1 = 62.8% (1,589/2,535), P/AI2 = 62.2% (583/937), P21 = 60.4% (1,568/2,595), P42 = 77.7% (2,000/2,567), and P84 = 90.5% (2,296/2,537). Herd and year were associated (both $P < 0.001$) with each reproductive outcome evaluated in the study, highlighting the importance of the herd differences and year-to-year environmental variation on reproductive performance in pasture-based dairy production systems.

CL Status

The incidence of cows that had a CL on the ovary at wk 3 and 7 was 45.5% (1,185/2,600) and 76.9% (1,879/2,445), respectively. There was no association between CL status at wk 3 and any reproductive performance variable (Tables 1 and 2). There was an association between CL status at wk 7 and SR21 ($P = 0.02$) and P21 ($P = 0.07$). Animals that did not have a CL at wk 7 were 0.5 (95% CI: 0.39–0.78) and 0.8 (95% CI: 0.64–1.03) times less likely to be inseminated and to establish a pregnancy during the first 3 wk of the breeding season, respectively, compared with cows that had a CL at wk 7 (Tables 1 and 2).

Uterine Health

Cows classified as having a uterine score of G3 at wk 7 had reduced likelihood of SR21 ($P = 0.03$; Table 3) compared with cows with a uterine score of G1. Cows with a uterine score of G4 at wk 3 had lower P/AI1 ($P = 0.09$; −7 percentage points) compared with cows in other URTS categories. Cows with uterine scores of G3 and G4 were less likely to achieve P21, P42, and P84 (Table 4), and the mean MSD-CI in cows with a uterine score of G4 was 4.8 ± 0.19 and 5.0 ± 0.24 d longer ($P = 0.07$) compared with cows with uterine scores of G1 and G2, respectively. Similarly, reproductive performance at the end of the breeding season and MSD-CI were associated with URTS at wk 7 (Table 4; Figure 2). Animals classified as having a uterine score of G4 had lower odds of P84 compared with cows with uterine scores of G1 and G2 (−12.4 and −6.4 percentage points, respectively; $P = 0.01$). The MSD-CI was longer ($P = 0.04$) in animals classified as having a uterine score of G4 compared with cows with uterine scores of G1, G2, and G3 (11.0 ± 0.2, 11.6 ± 0.18, and 10.8 ± 0.15 d extra, respectively; Figure 2).

When cows were classified as having normal or abnormal uterine health, an association between UHS at wk 3 and reproductive performance was observed (Tables 3 and 4). Cows with abnormal UHS at wk 3 had reduced odds of P21, P42, and P84 and MSD-CI was 2 ± 0.01 d longer ($P = 0.01$) compared with cows classified as having normal UHS. There were no associations between UHS at wk 7 and reproductive performance in the present study (Tables 3 and 4).

BCS and BCS Change

Table 5 summarizes the percentages of animals classified as having low, target, and high BCS at wk 3 and 7. Between wk 3 and 7, 4.0% (104/2,579) of cows were diagnosed as having lost ≥0.5 BCS unit, 32.1%

Table 1. Phenotypic associations at wk 3 and 7 postpartum between corpus luteum (CL) status and reproductive outcomes[1]

Variable	SR21 Served, % (no.)	SR21 OR[2] (95% CI)	SR21 P-value[3]	P/AI1 Pregnant, % (no.)	P/AI1 OR (95% CI)	P/AI1 P-value	P/AI2 Pregnant, % (no.)	P/AI2 OR (95% CI)	P/AI2 P-value
Wk 3									
CL present	95.6 (1,043/1,090)	Referent	0.56	62.1 (659/1,061)	Referent	0.81	63.5 (251/395)	Referent	0.72
CL absent	93.5 (1,219/1,303)	0.9 (0.65–1.32)		62.1 (790/1,277)	0.9 (0.77–1.10)		60.1 (291/484)	0.8 (0.62–1.14)	
Wk 7									
CL present	95.7 (1,771/1,849)	Referent	0.02	65.2 (1,119/1,714)	Referent	0.82	68.2 (422/618)	Referent	0.76
CL absent	93.6 (548/585)	0.5 (0.39–0.78)		65.0 (358/550)	1.0 (0.79–1.22)		67.6 (120/179)	1.0 (0.73–1.61)	

[1] SR21 = animals served in the first 21 d of the breeding season; P/AI1 = pregnant to first service; P/AI2 = pregnant to second service.

[2] Odds ratio.

[3] Significant differences when $P < 0.05$. Tendency when $P \geq 0.05$ and ≤ 0.10.

Table 2. Phenotypic associations at wk 3 and 7 postpartum between corpus luteum (CL) status and reproductive outcomes[1]

Variable	P21			P42			P84		
	Pregnant, % (no.)	OR[2] (95% CI)	P-value[3]	Pregnant, % (no.)	OR (95% CI)	P-value	Pregnant, % (no.)	OR (95% CI)	P-value
Wk 3									
CL present	59.1 (645/1,090)	Referent		78.6 (844/1,073)	Referent		90.4 (960/1,061)	Referent	
CL absent	59.3 (773/1,303)	1.0 (0.78–1.12)	0.51	78.1 (1,011/1,293)	1.1 (0.84–1.27)	0.71	91.0 (1,163/1,278)	1.1 (0.88–1.55)	0.23
Wk 7									
CL present	65.4 (1,151/1,758)	Referent		80.4 (1,397/1,736)	Referent		90.8 (1,559/1,717)	Referent	
CL absent	61.9 (344/555)	0.8 (0.64–1.03)	0.07	80.2 (445/553)	1.0 (0.78–1.33)	0.83	90.1 (496/550)	1.0 (0.70–1.43)	0.91

[1]P21 = pregnant within 21 d of the onset of breeding; P42 = pregnant within 42 d of the onset of breeding; P84 = pregnant within 84 d of the onset of breeding.
[2]Odds ratio.
[3]Significant differences when $P < 0.05$. Tendency when $P \geq 0.05$ and ≤ 0.10.

(829/2,579) lost 0.25 BCS unit, 51.7% (1,333/2,579) maintained constant BCS, and 12.1% (313/2,579) gained ≥0.25 BCS unit. There was no association between BCS change between wk 3 and 7 postpartum ($P > 0.05$) and any of the reproductive performance variables in the present study. A tendency for lower P84 ($P = 0.10$) and P/AI2 ($P = 0.06$) was observed in cows with low BCS at wk 3 (Supplemental Tables S1 and S2, https://doi.org/10.3168/jds.2018-16001). There were associations between BCS at wk 7 and P21, P42, P84 (Supplemental Table S1), and MSD-CI ($P = 0.01$). Cows with low BCS had lesser P21 (-7 percentage points), P42 (-9 percentage points), and P84 (-4 percentage points) compared with cows with target BCS. Moreover, cows with low and high BCS had longer MSD-CI (3.5 ± 0.26 and 2.1 ± 0.25 d extra, respectively) compared with cows with target BCS.

Metabolic Status, Parity, and Calving Date

The proportion of animals classified as having good, moderate, and poor metabolic status was 35.7% (818/2,294), 59.9% (1,376/2,294), and 4.4% (100/2,294) at wk 3 and 53.5% (1,243/2,323), 46.1% (1,070/2,323), and 0.4% (10/2,323) at wk 7, respectively. Lower P/AI1 was observed in cows classified as having poor metabolic status at wk 3 ($P = 0.10$) and moderate plus poor metabolic status combined at wk 7 ($P = 0.10$) compared with cows classified as having good metabolic status. Metabolic status was associated with P21 at both wk 3 ($P = 0.04$) and wk 7 ($P = 0.03$) postpartum. Cows classified as having poor metabolic status at wk 3 had lower P21 compared with those classified as having good and moderate metabolic status [(47.6% (40/84) vs. 60.2% (377/626) and 61.5% (654/1,062), respectively]. Similarly, animals classified as having good metabolic status at wk 7 had greater P21 compared with animals in the moderate plus poor categories combined [63.3% (600/947) vs. 57.0% (471/825), respectively].

There were associations between parity and calving date with reproductive performance outcomes of interest (Supplemental Tables S3 and S4, https://doi.org/10.3168/jds.2018-16001). Primiparous cows had greater P/AI1 (+3 percentage points), P/AI2 (+8 percentage points), P42 (+5 percentage points), and P84 (+2 percentage points) compared with second-parity cows. Cows in the late-calving category were 0.5 (95% CI: 0.37–0.90) and 0.7 (95% CI: 0.60–0.88) times less likely to achieve SR21 and P21, respectively, and had a longer MSD-CI (6.4 ± 0.2 and 4.6 ± 0.2 d; $P = 0.001$) compared with cows in the early- and mid-calving groups, respectively. Lower P84 was observed in late-calving cows compared with cows in the early- and mid-calving

Table 3. Phenotypic associations at wk 3 and 7 postpartum between ultrasound reproductive tract score[1] (URTS), uterine health status (UHS), and reproductive outcomes[2]

Variable	SR21			P/AI1			P/AI2		
	Served, % (no.)	OR[3] (95% CI)	P-value	Pregnant, % (no.)	OR (95% CI)	P-value	Pregnant, % (no.)	OR (95% CI)	P-value
URTS wk 3									
G1	92.3 (50/54)	0.8 (0.19–2.93)	0.62	63.3^{ab} (39/49)	1.0 (0.54–1.86)	0.09	60.0 (135/225)	0.9 (0.64–1.42)	0.3
G2	95.3 (609/639)	Referent		63.3^a (380/594)	Referent		65.0 (251/386)	Referent	
G3	93.0 (1,433/1,540)	0.9 (0.33–2.76)		62.3^{ab} (887/1,422)	0.9 (0.51–1.72)		66.4 (115/173)	1.1 (0.74–1.68)	
G4	91.9 (275/299)	0.8 (0.25–3.24)		55.5^b (151/272)	0.6 (0.2–0.98)		53.8 (7/13)	0.7 (0.15–2.7)	
UHS wk 3									
Normal[4]	95.5 (659/693)	Referent	0.16	63.7 (410/643)	Referent	0.34	63.8 (145/227)	Referent	0.44
Abnormal	92.8 (1,708/1,839)	0.7 (0.51–1.12)		61.2 (1,038/1,694)	0.9 (0.74–1.10)		60.8 (397/652)	0.8 (0.62–1.22)	
URTS wk 7									
G1	96.2^a (641/666)	Referent	0.03	62.1^a (376/605)	Referent	0.15	60.0 (135/225)	Referent	0.90
G2	95.2^{ab} (1,122/1,178)	0.7 (0.43–1.13)		64.9^a (714/1,100)	1.0 (0.84–1.30)		65.0 (251/386)	1.0 (0.74–1.52)	
G3	90.8^b (534/588)	0.4 (0.26–0.80)		67.0^a (360/537)	1.0 (0.8–1.4)		66.4 (115/173)	1.0 (0.64–1.58)	
G4	100^{ab} (22/22)	2.4 (0.14–42.66)		40.9^b (9/22)	0.3 (0.15–0.92)		53.8 (7/13)	0.8 (0.25–2.78)	
UHS wk 7									
Normal[5]	96.2 (641/666)	Referent	0.16	62.1 (376/605)	Referent	0.41	60.0 (135/225)	Referent	0.51
Abnormal	94.9 (1,678/1,768)	0.7 (0.45–1.11)		65.2 (1,083/1,659)	1.0 (0.88–1.32)		65.2 (373/572)	1.0 (0.74–1.47)	

a,b For each variable, different superscripts within a column indicate significant differences ($P < 0.05$).

[1] G1 = a typical spoke wheel–shaped lumen; G2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity; G3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity; G4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity.

[2] SR21 = animals served in the first 21 d of the breeding season; P/AI1 = pregnant to first service; P/AI2 = pregnant to second service.

[3] Odds ratio.

[4] Comprised cows classified as G1 and G2.

[5] Comprised cows classified as G1.

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Table 4. Phenotypic associations at wk 3 and 7 postpartum between ultrasound reproductive tract score[1] (URTS), uterine health status (UHS), and reproductive outcomes[2]

Variable	P21 Pregnant, % (no.)	P21 OR[3] (95% CI)	P21 P-value	P42 Pregnant, % (no.)	P42 OR (95% CI)	P42 P-value	P84 Pregnant, % (no.)	P84 OR (95% CI)	P84 P-value
URTS wk 3									
G1	54.0^ab (27/50)	0.6 (0.36–1.27)	0.03	78.0^a (39/50)	0.9 (0.45–1.95)	0.004	98.0^a (49/50)	2.7 (0.53–13.9)	0.01
G2	64.7^a (393/607)	Referent		81.3^a (488/600)	Referent		92.0^a (547/594)	Referent	
G3	58.6^b (855/1,458)	0.8 (0.65–0.99)		77.6^a (1,119/1,441)	0.7 (0.55–1.15)		90.3^a (1,285/1,423)	0.8 (0.57–1.12)	
G4	51.6^b (143/277)	0.6 (0.47–0.88)		68.9^b (189/274)	0.5 (0.38–0.76)		85.6^b (233/272)	0.5 (0.38–0.81)	
UHS wk 3									
Normal[4]	63.9 (420/657)	Referent	0.02	81.0 (527/650)	Referent	0.03	92.0 (595/647)	Referent	0.03
Abnormal	57.5 (998/1,735)	0.7 (0.65–0.96)		76.7 (1,308/1,705)	0.7 (0.61–0.98)		89.5 (1,518/1,695)	0.7 (0.50–0.97)	
URTS wk 7									
G1	60.2 (375/622)	Referent	0.33	78.6^a (483/614)	Referent	0.25	91.2^a (552/605)	Referent	0.01
G2	63.6 (715/1,124)	1.0 (0.80–1.15)		80.1^a (890/1,111)	0.9 (0.76–1.29)		91.8^a (1,013/1,103)	1.0 (0.71–1.44)	
G3	57.7 (355/545)	0.9 (0.71–1.23)		79.7^a (432/542)	0.8 (0.65–1.22)		89.3^ab (480/537)	0.6 (0.42–0.90)	
G4	45.4 (10/22)	0.4 (0.16–1.08)		63.6^b (14/22)	0.3 (0.15–0.97)		81.8^b (18/22)	0.2 (0.08–0.83)	
UHS wk 7									
Normal[5]	60.2 (375/622)	Referent	0.41	78.6 (483/614)	Referent	0.90	91.2 (552/605)	Referent	0.4
Abnormal	63.8 (1,080/1,691)	1.0 (0.88–1.33)		79.7 (1,336/1,675)	0.9 (0.77–1.25)		90.9 (1,511/1,662)	0.9 (0.64–1.25)	

a,b For each variable, different superscripts within a column indicate significant differences (P < 0.05).

[1] G1 = a typical spoke wheel–shaped lumen; G2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity; G3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity; G4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity.

[2] P21 = pregnant within 21 d of the onset of breeding; P42 = pregnant within 42 d of the onset of breeding; P84 = pregnant within 84 d of the onset of breeding.

[3] Odds ratio.

[4] Comprised cows classified as G1 and G2.

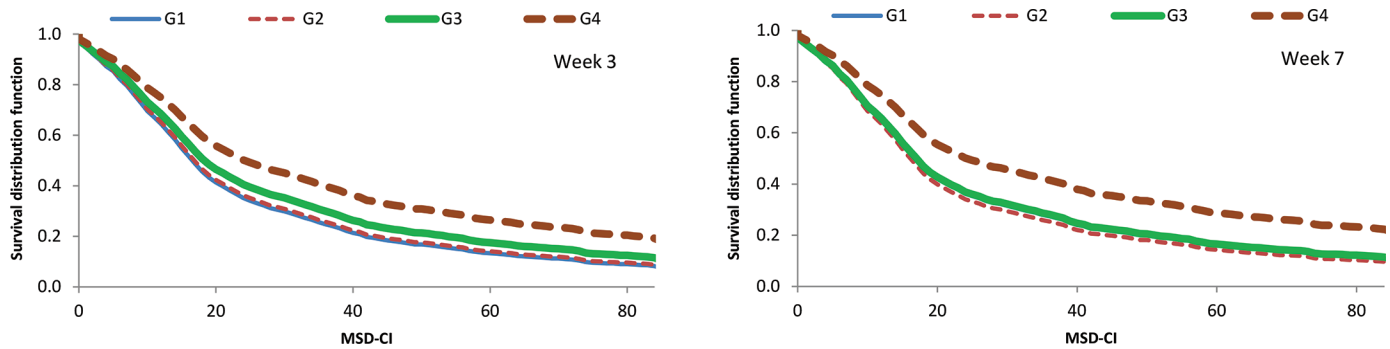[5] Comprised cows classified as G1.

Figure 2. Kaplan–Meier survival curve for associations between mating start date to conception interval (MSD-CI) and uterine reproductive tract score at wk 3 (left; $P = 0.07$) and wk 7 (right; $P = 0.04$). G1 = a typical spoke wheel–shaped lumen; G2 = a spoke wheel–shaped lumen with an enlarged center filled with a small volume (>2 and ≤5 mm diameter) of fluid of mixed echogenicity; G3 = a stellate-shaped lumen filled with a moderate volume (>5 and ≤10 mm diameter) of fluid of mixed echogenicity; G4 = a circular-shaped lumen filled with a large volume (>10 mm diameter) of fluid of mixed echogenicity. Cows classified as having a uterine score of G4 at both wk 3 and wk 7 had longer MSD-CI compared with cows classified as having uterine scores of G1 and G2.

categories (−3.1 and −2.5 percentage points, respectively; $P = 0.07$).

Genetic Traits

Fertility subindex, PTA for survival, and PTA for calving interval were strongly associated with reproductive performance outcomes of interest (Tables 6 and 7; Supplemental Tables S5 and S6, https://doi.org/10.3168/jds.2018-16001). Animals in the quartile with the greatest fertility subindex (i.e., >77) had greater odds of P/AI1, P/AI2, P21, P42, and P84 and had an MSD-CI that was 4.9 ± 0.04, 2.7 ± 0.03, and 2.6 ± 0.07 d shorter ($P < 0.0001$; Supplemental Figure S1, https://doi.org/10.3168/jds.2018-16001) compared with animals in the smallest and 2 intermediate quartiles for fertility subindex, respectively. Similarly, cows in the quartile with the smallest PTA for survival (i.e., ≤0.9) and cows in the quartile with the longest PTA for calving interval (i.e., >−1.2 d) had reduced odds of P/AI1, P/AI2 (Table 6), P21, P42, and P84 (Table 7) and had the longer MSD-CI (Figure 3) compared with cows in the other 3 quartiles. Cows in the 2 quartiles with the greatest PTA for milk kilograms (i.e., −2.5 to ≤109 and >109) had greater odds of P21 ($P = 0.04$) and P84 ($P = 0.007$) compared with cows in the other

2 quartiles. There was a tendency for reduced SR21 ($P = 0.06$) and P84 ($P = 0.10$) in cows in the quartile with the greatest PTA for milk protein percentage (i.e., >0.12%; Supplemental Tables S5 and S6, https://doi.org/10.3168/jds.2018-16001).

DISCUSSION

The present study investigated the associations between different postpartum phenotypes, genetic traits, and reproductive performance in seasonal-calving, pasture-based lactating dairy cows. Overall, the reproductive performance of the herds enrolled in the current study reached the fertility performance targets established for seasonal-calving, pasture-based lactating dairy cows (Butler, 2014): (1) ≥90% of lactating cows submitted for AI in the first 21 d of the breeding season, (2) 6-wk pregnancy rate of ≥70%, and (3) 12-wk pregnancy rate >90%. Hence, the study animals can be considered to have had good phenotypic fertility overall. It is important to note that this study used only a subpopulation of cows within each herd (first and second calvers, no clinical disease, and calving earlier than 30 d before the farm MSD) and likely represented the most fertile cows within the herd. Despite this, important associations were identified.

Table 5. Summary of prevalence (%; no. in parentheses) of cow BCS at wk 3 (14–27 DIM) and wk 7 (42–55 DIM) postpartum

BCS[1]	Wk 3 postpartum	Wk 7 postpartum	AI
Target	89.5 (2,327/2,599)	84.4 (2,066/2,445)	94.9 (2,409/2,538)
Low	8.6 (226/2,599)	14.5 (355/2,445)	4.6 (118/2,538)
High	1.7 (46/2,599)	0.9 (24/2,445)	0.4 (11/2,538)

[1]Evaluated by a single observer using a 1-to-5 scale (1 = emaciated; 5 = obese) with 0.25-point increments (Edmonson et al., 1989). Cows were classified according to their BCS as low (≤2.5), target (2.75–3.25), or high (≥3.5).

Table 6. Phenotypic associations between PTA for calving interval, PTA for survival, and reproductive outcomes[1]

Variable	SR21 Served, % (no.)	SR21 OR[2] (95% CI)	SR21 P-value	P/AI1 Pregnant, % (no.)	P/AI1 OR (95% CI)	P/AI1 P-value	P/AI2 Pregnant, % (no.)	P/AI2 OR (95% CI)	P/AI2 P-value
Calving interval			0.90			0.0004			0.08
≤−3.5	94.1 (645/685)	Referent		68.1a (428/628)	Referent		67.6a (134/198)	Referent	
>−3.5 to ≤−2.25	93.5 (648/685)	0.88 (0.56–1.37)		62.5b (403/644)	0.7 (0.60–0.98)		63.0a (159/252)	0.8 (0.36–1.26)	
>−2.25 to ≤−1.2	94.8 (569/600)	0.9 (0.61–1.60)		61.7b (341/552)	0.7 (0.56–0.93)		62.5a (130/208)	0.7 (0.45–1.11)	
>−1.2	94.5 (626/662)	0.9 (0.58–1.49)		57.9b (350/604)	0.5 (0.45–0.74)		56.6b (136/239)	0.5 (0.46–0.89)	
Survival[3]			0.90			0.002			0.01
≤0.9	94.3 (621/658)	Referent		56.3a (334/593)	Referent		57.6a (150/260)	Referent	
>0.9 to ≤1.46	94.9 (606/638)	1.0 (0.65–1.71)		62.9a (373/593)	1.2 (0.98–1.59)		57.1a (124/217)	1.0 (0.72–1.58)	
>1.46 to ≤2	94.0 (587/624)	1.0 (0.64–1.59)		66.5b (392/589)	1.5 (1.21–1.99)		66.8b (151/226)	1.6 (1.11–2.51)	
>2	93.5 (666/712)	0.9 (0.56–1.45)		65.0b (423/650)	1.4 (1.13–1.84)		67.5b (131/194)	1.6 (1.08–2.51)	

[a,b]For each variable, different superscripts within a column indicate significant differences ($P < 0.05$).

[1]SR21 = animals served in the first 21 d of the breeding season; P/AI1 = pregnant to first service; P/AI2 = pregnant to second service.

[2]Odds ratio.

[3]Longevity in the herd.

Table 7. Phenotypic associations between PTA for calving interval, PTA for survival, and reproductive outcomes[1]

Variable	P21 Pregnant, % (no.)	P21 OR[2] (95% CI)	P21 P-value	P42 Pregnant, % (no.)	P42 OR (95% CI)	P42 P-value	P84 Pregnant, % (no.)	P84 OR (95% CI)	P84 P-value
Calving interval			0.003			0.0003			0.0009
≤−3.5	63.1a (408/646)	Referent		82.2a (525/638)	Referent		93.3a (587/629)	Referent	
>−3.5 to ≤−2.25	62.3a (408/655)	0.8 (0.65–1.04)		78.0a (507/650)	0.7 (0.56–1.00)		91.3a (588/644)	0.8 (0.56–1.29)	
>−2.25 to ≤−1.2	62.4a (352/564)	0.8 (0.68–1.14)		78.7a (440/559)	0.7 (0.54–1.00)		90.9a (502/552)	0.8 (0.53–1.25)	
>−1.2	57.8b (358/619)	0.6 (0.53–0.88)		73.2b (448/612)	0.5 (0.39–0.70)		87.1b (527/605)	0.5 (0.38–0.85)	
Survival[3]			0.0002			<0.0001			0.0005
≤0.9	53.4a (329/615)	Referent		70.9a (429/605)	Referent		86.2a (515/597)	Referent	
>0.9 to ≤1.46	64.9b (391/602)	1.4 (1.16–1.91)		80.2b (479/597)	1.6 (1.29–2.13)		90.7b (539/594)	1.5 (1.10–2.2)	
>1.46 to ≤2	61.1b (410/670)	1.5 (1.23–2.01)		79.0b (526/665)	1.7 (1.33–2.33)		92.9b (604/650)	2.0 (1.37–2.98)	
>2	69.2b (379/547)	1.5 (1.22–2.01)		82.0b (486/592)	1.9 (1.41–2.54)		92.6b (546/589)	1.9 (1.32–2.88)	

[a,b]For each variable, different superscripts within a column indicate significant differences ($P < 0.05$).

[1]P21 = pregnant within 21 d of the onset of breeding; P42 = pregnant within 42 d of the onset of breeding; P84 = pregnant within 84 d of the onset of breeding.

[2]Odds ratio.

[3]Longevity in the herd.

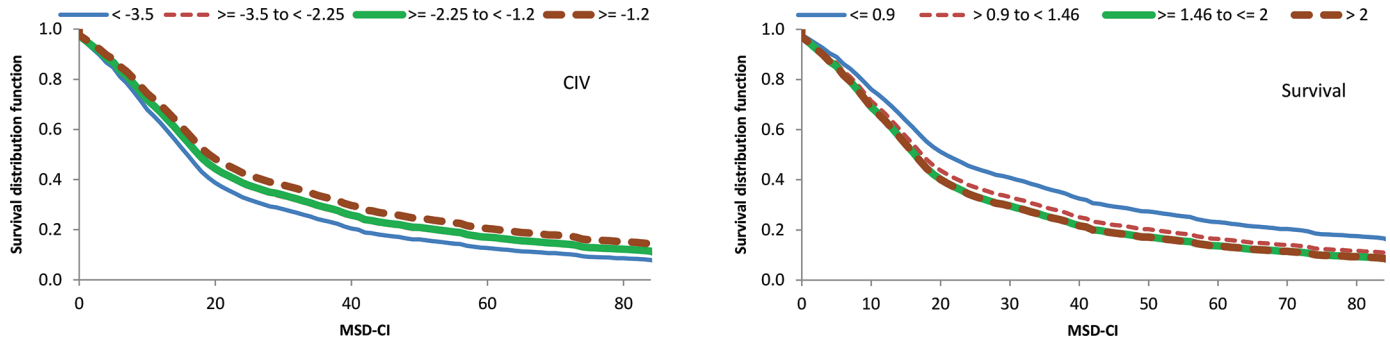Rojas Canadas et al.: POSTPARTUM PHENOTYPES, GENETIC TRAITS, AND REPRODUCTIVE PERFORMANCE

Figure 3. Kaplan–Meier survival curve for associations between mating start date to conception interval (MSD-CI) and PTA for calving interval (CIV; left; $P = 0.008$) and PTA for survival (longevity in the herd; right; $P = 0.008$). Cows in the quartiles with the 2 longest PTA for calving interval and in the quartile with the smallest PTA for survival had longer MSD-CI compared with the other 2 quartiles for calving interval and the other 3 quartiles for survival, respectively.

Ovulatory Ovarian Activity

Ever since Thatcher and Wilcox (1973) stated that the occurrence of multiple estrous cycles during early lactation was associated with increased conception rate, the early resumption of ovulatory ovarian activity has been considered beneficial to fertility. Nevertheless, it is still debated whether early resumption of ovulatory ovarian activity enhances reproductive performance in dairy cows (Smith and Wallace, 1998; Galvão et al., 2010; Bittar et al., 2014). In the present study, cows that had a CL at wk 7 postpartum were more likely to be inseminated during the first 21 d of the breeding period compared with cows that did not have a CL, but this association was not observed at wk 3 postpartum. Moreover, there was a tendency for greater P21 in cows that had a CL at wk 7 postpartum compared with cows that did not have a CL. Nevertheless, presence of a CL at wk 3 or 7 postpartum was not associated with other reproductive performance outcomes of interest (P42, P84, P/AI1, P/AI2), which is in agreement with the findings of Sakaguchi et al. (2004). Conversely, Santos et al. (2009) and Vieira-Neto et al. (2014) reported greater conception rates at 30 and 58 d post-AI, greater pregnancy rate at first AI, and shorter calving to pregnancy interval, respectively, in cows that had resumed ovarian cyclicity at 35 and 65 d postcalving compared with cows that had not resumed ovarian cyclicity at these time points. Timing of examinations differed between studies, which likely explains, at least in part, some of the different findings in these studies.

Uterine Health

There is general consensus in the literature that endometritis diminishes reproductive performance in cattle (LeBlanc et al., 2002). Endometritis has a del-

eterious effect on sperm survival and sperm function (Lonkar and Dedon, 2011), oocyte and embryo development (Hill and Gilbert, 2008; Cerri et al., 2009), and implantation (Kodaman et al., 2004). In the present study, 72.7 and 72.0% of animals had abnormal UHS at wk 3 (G3 and G4) and wk 7 (G2, G3, and G4), respectively. Ribeiro et al. (2013; 15.5%) and Mee et al. (2009; 29.0%) reported a lesser proportion of cows with clinical endometritis and abnormal UHS, respectively, but again the methods and timing of endometritis diagnosis differed between studies.

The results arising from this study highlight the strong association between uterine health and reproductive performance, in agreement with previous reports (LeBlanc, 2008; Gilbert, 2011). We observed a strong association between URTS at both wk 3 and wk 7 and reproductive performance, indicating that ultrasound evaluation of the reproductive tract is a useful tool to predict likelihood of pregnancy failure in dairy cows. It should be noted that there were no differences in reproductive performance between cows diagnosed as having uterine scores of G1 and G2 at both wk 3 and wk 7. Hence, in the present study, deleterious effects on reproductive performance occurred only in cows diagnosed as having uterine scores of G3 or G4. These findings are in agreement with McDougall et al. (2007) and Mee et al. (2009). When cows were classified as having abnormal UHS at wk 3 (URTS G3 and G4), they had reduced odds of pregnancy establishment during the breeding season (P21, P42, P84) and had a longer MSD-CI compared with cows diagnosed as having normal UHS (URTS G1 and G2), in agreement with McDougall et al. (2007). It is likely that an extended period of subclinical endometritis occurs in cows that were previously diagnosed as having clinical endometritis during the postpartum period despite resolving the clinical problem (Sheldon et al., 2009).

Prolonged subclinical endometritis is associated with reduced reproductive performance (Madoz et al., 2013). Of note, however, we did not observe a difference in reproductive performance in cows diagnosed with abnormal UHS (URTS G2, G3, and G4) compared with cows with normal UHS (G1) at wk 7. This reflects the relatively lower incidence of G3 and G4 URTS at wk 7 compared with wk 3 and the lack of difference in reproductive performance between cows diagnosed as G1 or G2 at wk 7.

BCS

Body condition score at calving and at AI and BCS change have previously been described as risk factors affecting reproductive performance in both confinement (see meta-analysis by López-Gatius et al., 2003) and pasture-based systems (Buckley et al., 2003; Roche et al., 2007; Herlihy et al., 2013). In the current study, cows with low BCS at both wk 3 and wk 7 postpartum had poorer reproductive performance compared with cows with target BCS, in agreement with previous reports (Buckley et al., 2003; Santos et al., 2009; Ribeiro et al., 2013). Nevertheless, BCS at AI and BCS change between wk 3 and 7 postpartum were not associated with any reproductive performance variable in the present study, in contrast to previous reports (Roche et al., 2007; Santos et al., 2009). These differences between studies can be explained by differences in the proportion of cows with no change in BCS [52.2% (1,326/2,538) in the current study vs. 16.4% (1,008/6,124) in Santos et al., 2009] and time of measurement of BCS change (between wk 3 and 7 postpartum in the present study vs. between calving and first AI in Santos et al., 2009).

Metabolic Status, Parity, Calving Date, and Reproductive Performance

The severity of negative energy balance (**NEBAL**) influences the concentration of blood metabolites, including FA (Ospina et al., 2013), BHB (McArt et al., 2013), and glucose (Butler, 2003). Postpartum blood concentrations of FA, BHB, and glucose have been used to predict reproductive performance in previous studies (Ospina et al., 2010a; Garverick et al., 2013). In the current study, cows classified as having poor metabolic status at both wk 3 and wk 7 postpartum had reduced odds of pregnancy establishment at the beginning of the breeding period (SR21, P/AI1, and P21) compared with the other 2 categories. These findings are in agreement with Ospina et al. (2010b) and Cardoso et al. (2013). The critical window for follicle exposure to NEBAL may be 6 to 12 wk before ovulation (Garverick et al., 2013). Britt (1994) suggested that adverse

events that occur during early follicular development are associated with poor subsequent fertility when cows are inseminated at 50 to 100 d postpartum. Moreover, Wathes et al. (2011) reported that cows exposed to severe NEBAL experience changes in insulin and IGF-1 signaling pathways in the postpartum endometrium, affecting the rate of tissue repair with likely adverse effects on subsequent fertility.

Overall, second-parity cows had poorer reproductive performance than primiparous cows in the present study. This observation might be explained by lower milk yield (Santos et al., 2009) and lower pregnancy loss (Santos et al., 2009) reported in primiparous compared with second-parity cows. Calving date has been reported as an important factor affecting both production (Dillon et al., 1995) and reproductive performance (Garcia and Holmes, 1999) in pasture-based, seasonal-calving systems. In this study, cows in the late-calving category had poorer reproductive performance compared with cows in the early- and mid-calving categories, in agreement with previous reports (Garcia and Holmes, 1999; Herlihy et al., 2013; Sánchez et al., 2018). This reflects the reduced time available to complete postcalving uterine involution, resolve uterine infection, and resume normal estrous cyclicity before the fixed calendar MSD in late-calving cows, as these are all factors that affect reproductive performance (McDougall et al., 2007; Mee et al., 2009; Gautam et al., 2010).

PTA and Fertility Subindex

Recent studies from our group examining the genetic merit for fertility traits have reported greater DMI and BCS, superior metabolic status and uterine health (Cummins et al., 2012a; Moore et al., 2014a; Moran et al., 2017), earlier resumption of ovarian cyclicity, and greater luteal phase circulating concentrations of progesterone (Cummins et al., 2012b; Moore et al., 2014b) in cows with good genetic merit for fertility traits compared with cows with poor genetic merit for fertility traits. The results arising from this study highlight the strong associations between fertility subindex and PTA for both calving interval and survival with reproductive performance, in agreement with Fenlon et al. (2017) and Hempstalk et al. (2015). This highlights the importance of selecting for fertility traits and long-term gains that can be achieved in herd phenotypic reproductive performance.

Phenotypic milk protein concentration has been positively associated with a range of measures of reproductive performance in both seasonally calving herds (Patton et al., 2007; Morton et al., 2016) and year-round-calving herds (Miettinen and Setälä, 1993). In the current study, a tendency for reduced odds of

pregnancy establishment was observed at the last stage of the breeding period (P84) for animals in the quartile with the greatest PTA for milk protein percentage. This observation of an unfavorable association between PTA for milk protein percentage and fertility performance is not consistent with previous studies that demonstrated a favorable association between phenotypic milk protein percentage and fertility performance (Buckley et al., 2003; Morton et al., 2017). It is possible that phenotypic milk protein percentage is reflective of both genetic merit for milk protein percentage and environmental influences related to animal nutritional status. On the other hand, there was a positive association between genetic merit for milk (kg) and reproductive performance, in agreement with phenotypic outcomes reported by others (Berry et al., 2003; Buckley et al., 2003; Morton et al., 2016). Cows in the 2 greatest quartiles for milk (kg) had greater P21 and P84 compared with cows in the other 2 quartiles.

A companion paper from this study reported that PTA for calving interval and survival was associated with uterine health and CL status, but a similar relationship was not observed for fertility subindex (Rojas Canadas et al., 2020). The results arising from this study, however, clearly highlight the strong associations between uterine health, CL status, fertility subindex (and its component traits PTA for calving interval and survival), and reproductive performance. Strong effects on pregnancy establishment were observed within the range of fertility subindex values encountered in the current study, highlighting the long-term gains achievable through selecting for fertility traits.

CONCLUSIONS

The present study clearly demonstrates that postpartum reproductive phenotypes, bioenergetic status, fertility subindex, and PTA for calving interval, survival, milk (kg), and protein in milk (%) were all associated with reproductive performance. Ultrasound evaluation of the reproductive tract at wk 3 and 7 postpartum identified an unfavorable association between G3 and G4 uterine scores and reproductive performance. Cows in the quartiles with the greatest fertility subindex, PTA for survival, and milk (kg) and in the quartile with the shortest calving interval had superior reproductive performance compared with cows in the other quartiles for each of these variables. Achieving target BCS, good metabolic status, and presence of a CL by wk 7 postpartum, minimizing the incidence of animals having uterine tract scores of G3 and G4, and selecting animals based on fertility subindex (and its component genetic traits) were all associated with improved repro-

ductive performance in seasonal-calving, pasture-based dairy cows.

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