Relative effect of milk constituents on fertility performance of milk-recorded, spring-calving dairy cows in Ireland

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ABSTRACT

Several studies have reported associations between milk composition data and fertility performance. However, no work to date has estimated the effect of milk constituents on fertility performance in cows with low milk constituent concentrations. The objective of this study was to assess the association between milk constituents, animal characteristics, and time from mating start date (MSD) to conception using survival analysis. Furthermore, we aimed to investigate the relative effect of each variable by predicting median times to conception for animals with different combinations of characteristics and milk compositions. The final data set consisted of 87,227 cow lactation records from 64,519 cows in 2,049 herds with calving dates from 2010 to 2013. Milk recording data from each lactation were used, including test day recordings at 0 to 30, 31 to 60, and 61 to 90 d in milk (DIM). The analysis was limited to spring-calving cows (i.e., animals calving from January to May inclusive). Mating start date was determined for each unique herd in each year. A cow-specific MSD (MSD_{cow}) was defined taking into consideration the MSD for each herd and the calving date and a minimum calving to insemination interval of each herd year. The conception date for each cow was estimated using the subsequent calving date. Cows with no subsequent calving date were assumed not to have conceived. Time from MSD_{cow} to approximate conception date was analyzed using survival analysis. Cox proportional hazard models were constructed for each of the 3 recording windows: 0 to 30, 31 to 60, and 61 to 90 DIM. A fourth model was used to assess the dynamics of milk composition over the 3 windows. To investigate the effect of these variables, model outputs were used to create parametric accelerated failure time models to predict median survival times for animals at the 10th and 90th percentiles of the variable of interest

but otherwise identical across the rest of the variables. Results demonstrated that fertility breeding subindex had the largest effect on time from MSD_{cow} to conception, with an additional 62 d open for those in the 10th percentile versus those in the 90th percentile. Of the milk constituents, milk lactose concentration had the greatest effect on MSD to conception, particularly when measured from 0 to 30 DIM. An additional 10 d open resulted from comparing those in the 10th and 90th percentiles. Milk protein concentration, although statistically significant, had a lower effect on fertility outcome when comparing cows in the 10th and 90th percentiles for this exposure variable. The greatest effect was found in the 61 to 90 DIM recording window, where cows in the 10th percentile had an additional 9 d open at the subsequent breeding season compared with those in the 90th percentile. Overall, our study shows that although the associations between milk constituents and fertility are statistically significant, their overall influence in determining MSD to conception in this study population is relatively modest, particularly compared with fertility breeding subindex, when comparing cows at the 10th and 90th percentiles. Of the milk constituents measured, milk lactose concentration measured at 0 to 30 DIM had the greatest effect in determining fertility outcome when comparing cows at the 10th and 90th percentiles. The predictive value of early-lactation test day milk composition data on hazard of pregnancy during the following breeding period, within a spring-calving context, appears to be relatively modest at the individual-cow level. Further work is required to test the usefulness of these associations at the herd level.

Key words: milk protein, milk lactose, seasonal-calving herd, milk composition

INTRODUCTION

Milk composition data have been widely used as an indicator of energy balance. Milk protein concentration is reduced in association with an energy deficit experienced in negative energy balance (Friggens et

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al., 2007), whereas milk fat concentration may increase with excessive BCS loss or mobilization (Bauman and Griinari, 2003). Consequently, both low milk protein percentage (Fulkerson et al., 2008) and milk fat:protein ratio greater than 1.4 (Heuer et al., 2000) have been used as indicators of excessive negative energy balance in early lactation. However, there is some debate over the usefulness and reliability of using milk constituent data to predict energy balance, particularly at the individual-cow level (Hudson and Green, 2018).

In Ireland, milk is produced predominantly by pasture-based, seasonal, spring-calving herds (Dillon et al., 2008). However, some farmers may also calve a smaller proportion of the herd in the autumn, often called split calving. The number of split-calving herds has decreased over time. The main goal of reproductive management in seasonal, spring-calving herds is to have a high proportion of cows conceive within a restricted period to maintain herd size and calving pattern. Compact seasonal-calving systems ensure that maximum numbers of cows are in peak lactation coincident with peak grass growth (Roche et al., 2007; Ramsbottom et al., 2015). Concentrated calving demands excellent fertility during the breeding season, with most of the herd becoming pregnant during a restricted breeding period commencing at mating start date (MSD). The MSD for each herd is decided by the individual farm, but the goal is that >90% of the herd is submitted within 3 wk of MSD and ultimately that >70% of the herd is pregnant after 6 wk of MSD (Butler, 2014).

Several studies have assessed the relationship between early-lactation milk recording data and fertility using various measurements of reproductive performance. Comparisons between studies are difficult when different measures of milk constituent data are used, such as early-lactation averages versus actual test day data. Similarly, different measures of fertility performance are used across different studies, and therefore direct comparisons can be challenging. Milk protein concentration has been positively associated with reproductive performance in all production systems across several studies (Fahey et al., 2017; Morton et al., 2018). The association between milk protein concentration and fertility has been reported as improved submission and conception rates and better pregnancy rates at the end of the breeding season (Morton et al., 2016). In an Irish study, cows with a higher milk protein percentage and higher protein:fat ratios at the start of breeding were more likely to be submitted for breeding within the first 21 d of the breeding season. Positive effects on conception rate were also identified for the highest protein yields, even when genetics were accounted for (Buckley

et al., 2003). Morton et al. (2016) showed that when accounting for milk yield, a positive relationship between milk protein concentration in the first 120 DIM was associated with multiple positive reproductive outcomes, including 3-wk submission rate and 6-wk in-calf rate. These measures are commonly used as key performance indicators in seasonal-calving systems. In addition, cows producing lower milk protein concentrations were more likely to fail to conceive or maintain pregnancy (Morton et al., 2016).

Traditionally, it has been assumed that the relationship between milk constituents and fertility performance is reflective of negative energy balance, which affects both. However, more recently it has been shown that this relationship is likely more complex than previously thought and that the relationship may be influenced by both genetic and nongenetic components, such as nutritional and environmental factors (Morton et al., 2017). Importantly, the magnitude of effect estimates between milk constituents and subsequent fertility is likely to vary with stage of lactation (Madouasse et al., 2010). For example, Morton et al. (2016) showed beneficial effects of milk protein concentration on reproduction, with the largest effect at 31 to 60 DIM. Therefore, the effect estimates of milk constituents on hazard of pregnancy during the following breeding period must account for the stage of lactation (or DIM) when the milk constituents were measured.

However, although these studies demonstrate statistically significant associations between these constituents and fertility performance, there has been little work on their practical implications relative to each other and relative to other variables affecting fertility, such as genetics and parity. In addition, the majority of the previous literature in this area has focused on summarizing data across groups of animals in terms of classical fertility key performance indicators, such as submission and conception rate. In all seasonal- or block-calving systems, where the goal is to have a high proportion of cows conceive within a restricted period of calendar time, time taken from MSD to conception is important as a primary measure of fertility performance. Therefore, the objective of this study was to use survival analysis to investigate the association between test day milk constituent variables, genetic variables, and parity and whether the cow conceived, as well as the time from MSD taken for animals to conceive. A second objective was to compare test day milk constituent variables as well as genetic variables and parity by predicting for each variable the effect of replacing a cow in the 10th percentile for that variable with an otherwise identical cow in the 90th percentile.

MATERIALS AND METHODS

Data

This study was conducted entirely from milk recording data; no culling records or records of insemination or pregnancy data were available. The population of interest was spring-calving cows. Dairy herds in Ireland are predominantly seasonally calving. However, some seasonal-calving herds in Ireland calve most of the herd in spring and the remainder in autumn (Berry et al., 2013); these are generally termed split-calving herds. This practice may affect the fertility performance of those herds because it facilitates “recycling” of cows into subsequent breeding blocks. Because our population of interest was spring-calving cows, our analysis was conducted on spring-calving cows from spring- and split-calving herds from which our findings are reported. However, as an additional step, the final models were also applied to a subset of animals coming from spring-calving herds only to investigate whether differences existed in the observed associations depending on herd selection.

Milk records from the years 2010 to 2014 were obtained for all individual cows from the Irish Cattle Breeding Federation (Co. Cork, Ireland) database with a main breed Holstein or Friesian. Test day records included milk yield, fat, and protein yield and percentages; log-transformed SCC; cow breed; breeding values as measured by economic breeding index (EBI), including subindices; and PTA values for relevant traits (milk yield, milk fat, and protein percentages). The EBI is expressed as expected profit per lactation of the progeny of the animal in question, and the genetic merit values of the component traits are expressed as PTA (Ramsbottom et al., 2015). The EBI used in Ireland is a single-figure profit index used for breeding. It is a weighted figure currently made of 7 subindices: production (33%), fertility (35%), calving (10%), beef (8%), maintenance (7%), management (3%), and health (4%; Berry et al., 2007). The fertility subindex is made up of traits based on calving interval and survival. The calving subindex consists of information about calving difficulty, gestation length, and calf mortality. The management subindex comprises the traits milking duration and temperament (Berry et al., 2007). The genetic data for each cow consisted of the genetic evaluation of that cow at the point of retrieval.

Data analysis and visual presentation of results were performed in RStudio (R Core Team, 2017) using the “dplyr” (Wickham et al., 2018), “data.table” (Dowle et al., 2014), “survival” (Therneau and Grambsch, 2000), and “flexsurv” (Jackson, 2016) packages. First, the

date of conception was estimated for each cow as 282 d before the subsequent calving date for that animal (Fitzgerald et al., 2015). Next, edits were performed identical to those described previously (Carty et al., 2017). Briefly, data were analyzed for outliers, and records missing test day milk yield or milk composition data were removed. Records that were missing EBI, herd identification, or test date were removed from the data set. Test day records that were outside ± 3 standard deviations from the mean of test day milk yield, test day milk fat or protein yield, or test day milk fat or protein concentration were omitted. In addition, records of test day milk yields < 4 kg and records for animals of parities > 10 were removed. Only cows of Holstein and Friesian breeds were retained in the data set. Days in milk was calculated for all cows by subtracting the recorded calving date from the sampling date for each record, and all records < 1 and > 305 DIM were omitted. Only animals with a test day recording in each of the 3 recording windows (0–30, 31–60, and 61–90 DIM) were retained for the final analysis. A detailed description of all data edits is included in Supplemental File S1 (<https://doi.org/10.3168/jds.2018-15490>).

Fertility data for this study were constructed using calving dates (from spring-calving lactation records) reported in the milk recording data. The fertility measure used was the point at which the cow became at risk of pregnancy (MSD_{cow}) to conception, which was approximated as follows. The conception date for each cow in each lactation was estimated using the subsequent calving date (i.e., 282 d before the calving date of each cow’s next lactation; Fitzgerald et al., 2015). The MSD was determined for each herd, each year, as the first conception date within that calving season (MSD_{herd}). Herds with an MSD outside of April or May were discarded from the data set. Records from herds with fewer than 20 cows at this point were dropped. To avoid errors in the estimation of the MSD, the MSD was estimated before major data edits such as restricting the data to cows with sufficient milk recordings. In addition, cows with very early conception dates (DIM < 20) were not used to estimate the MSD. Next, for each individual cow, the MSD_{cow} was defined. The MSD_{cow} considers both the MSD_{herd} and a herd-specific minimum calving to insemination interval (MCII) of the cow, such that the individual MSD_{cow} was equal to the MSD_{herd} except in those cases where the cow calved close to or after the MSD_{herd} (i.e., cows whose MCII had not ended by MSD_{herd}). In those cases, the cow was assumed to be not at risk of pregnancy while already in calf or very early in lactation. For example, for a cow 50 DIM at the MSD_{herd} , the MSD_{cow} was equal to the

MSD_{herd} because MCII was always ≤ 42 d. However, for a cow that had not yet calved at the MSD_{herd} , the MSD_{cow} was defined as the calving date of the cow plus an MCII defined at individual herd-year level.

The MCII was defined individually for each herd each year and was calculated by assessing the lowest DIM in which a conception occurred for that herd in each year, up to a maximum of 42 d. Cows conceiving ≤ 20 DIM were discarded from the data set and were not used to define the herd MCII. The length of the breeding season was defined according to the time between the MSD_{herd} and the last conception date in the herd year. For each of the lactations, cows without an estimated conception date were right censored at $MSD_{herd} + 100$ d unless the breeding season length was < 100 d, in which case $MSD_{herd} + \text{breeding season length}$ was used as the censoring time point. The analysis was limited to spring-calving cows (i.e., animals calving from January to May inclusive). Only herd breeding periods that were greater than 6 wk in duration with MSD of April or May and with at least 20 cows calving in that spring-calving season were included in the analysis.

The MSD_{cow} to conception intervals were calculated as conception date minus $MSD + 1$, such that cows conceiving on the MSD were given a value of 1 d. All cows present in the herd with a calving date recorded for that spring-calving season (January to May inclusive) in each year were presumed to be eligible to conceive until they were right censored. This study population included cows that did not recalve and those that did recalve. This decision assumes that all animals that calved in the herd were at risk of conception up until $MSD_{herd} + \text{breeding season length}$ (to a maximum of 100 d). No culling data were available for these animals that might facilitate censoring at an earlier stage. However, because the data set included only cows that had 3 milk recordings in the first 90 DIM, all cows enrolled in the study were present in the herd for at least 3 mo after calving. The target breeding season length for Irish seasonally calving dairy herds is 12 wk (Butler, 2014). However, examination of the distribution of breeding season lengths demonstrated that many herds in reality bred for longer than this. For this study, we used the last conception date in the season to determine the length of the breeding season for each herd up to a maximum of 100 d.

Statistical Analysis

The data were analyzed using a Cox proportional hazards survival analysis accounting for herd as a frailty effect and all relate to fertility performance during

the following breeding period. The predictive value of early-lactation test day milk composition data on hazard of pregnancy during the following breeding period was analyzed. The dependent variable in all analyses was MSD_{cow} to conception. Separate models were constructed for each of the 3 milk recording windows: 0 to 30, 31 to 60, and 61 to 90 DIM, designated as models 1, 2, and 3, respectively. A fourth model, called model 4, was used to model the longitudinal interpretation of the milk recording data over all 3 windows. Finally, to assess the outputs from each model, 4 accelerated failure time models were constructed from the outputs of models 1 to 4 and used to predict time from MSD_{cow} to conception for cow lactation records with specific characteristics and milk recording results. Days in milk at MSD_{cow} was forced into all models as both linear and second-order polynomial terms, and calving year was included as a stratified effect in all models.

Cox Models: Models 1 to 3. To ensure that variables met the time dependency assumptions of the model, each variable was first assessed for time dependency through visual appraisal of Kaplan–Meier plots and log–log plots. Each variable was then tested individually for significance accounting for herd as a frailty effect, DIM at MSD , and calving year. For continuous variables, the relationship between the variable of interest and the time from MSD_{cow} to conception was first plotted. Variables that visually appeared to have a curvilinear relationship with MSD_{cow} to conception were assessed in the univariable analysis as both linear and second-order polynomial terms. The form resulting in the best model fit was used in the final model. Variables with $P < 0.2$ in the univariable analysis were brought forward to the multivariable analysis. The multivariable models were constructed individually using a forward stepwise approach. Variables were first screened in a univariable analysis; each variable was then added to the multivariable model in order of their univariable Wald P -value, with variables with the lowest P -value added first. Variables with Wald $P < 0.05$ were retained in the multivariable model, including categorical variables and continuous variables fitted as both linear and second-order polynomials. After the addition of each new variable, P -values for the remaining variables in the model were recalculated. Variables with a P -value > 0.05 were removed from the model before further variables were added. After the addition of each new variable, Wald P -values for the remaining variables in the model were recalculated. Before adding each variable, correlation between variables was assessed using the Pearson correlation coefficient; for highly correlated variables (> 0.7), only 1 variable was

chosen for inclusion in the final model. The decision of which variable to include was made based on which variable resulted in the best overall model fit as assessed by the Akaike information criterion (AIC). For moderately correlated variables, if the inclusion of both variables resulted in nonsignificance when both were included but the inclusion of either was significant individually, then the best variable was selected by comparing the AIC of the 2 potential models. These variables, as well as other variables that dropped out during the model construction, were offered to the final model. Finally, all significant 2-way interactions between the final set of variables were included in the final model.

Cox Models: Model 4. Model 4 assessed the dynamics of the milk constituents over all 3 recording windows. Milk constituents (fat, protein, and lactose concentrations) were characterized in several ways. First, in each recording window cows were determined as being low (less than the 33rd percentile) or medium-high (greater than the 33rd percentile) for each of the 3 constituents. A categorical variable was then created for each constituent for each cow based on their transition between these categories across the 3 windows. The mean and minimum values over the 3 windows were also calculated. Only 1 summary value for each constituent was used in the final multivariable model, and all continuous variables were trialed as linear and quadratic forms. The summary value used for each constituent was selected based on comparison of the AIC of the respective models. For each of the models, hazard ratios were calculated as the exponential of the coefficients.

Accelerated Failure Time Models. Each of the final Cox models were brought forward into a parametric accelerated failure time model. Clustering was accounted for by including herd as a frailty effect. For each model, a range of distributions were trialed: Weibull, exponential, logistic, log-logistic, log-normal, and Gaussian. An additional natural cubic spline model was trialed, although this model did not facilitate the incorporation of frailty or strata. The distribution was selected based on comparison of the AIC values and visual comparison with the null Kaplan-Meier model. These models were used to compare the effect of variables of interest by predicting the survival functions of animals with a given combination of variables. For continuous variables, the survival function was plotted for animals at the 10th and 90th percentiles of the variable with all other variables held at the median for the study population. Similarly, for categorical variables, survival functions were plotted for the categories at the extremes.

RESULTS

Descriptive Statistics

The initial data set included 11,935,017 milk records from 1,119,710 unique cows in 7,726 unique herds. The median herd size, defined as the number of cows calving in the herd in a calendar year, increased from 63 in 2010 to 72 in 2014; the proportion of those cows that had a subsequent calving date in the data set was 0.78 in 2010 and 0.71 in 2013.

After data edits and removing missing values and outliers (Supplemental File S1; <https://doi.org/10.3168/jds.2018-15490>), the final data set consisted of 87,227 cow lactations from 64,519 cows in 2,049 herds. Of those cows calving in 2010, 79% calved subsequently in the data set compared with 75% in 2013. For those with a subsequent calving date, the estimated conception date (as a proportion of the overall cow lactations rather than as a proportion of only cows that conceived) was within 42 d of MSD_{cow} for 42% of lactations in 2010 and 45% of lactations in 2013. Of the remaining lactations, the subsequent calving date was between January and May of the following year for 25% of cow lactations in both 2010 and 2013 but with an estimated conception date that was greater than 42 d from MSD_{cow}. The subsequent calving date was between June and December of the following year for 8.1% of lactations in 2010 and 5.5% of lactations in 2013; between January and May 2 yr after the study lactation for 3.3% of lactations in 2010 and 2.7% of lactations in 2012 (because not enough time had elapsed to count 2013); and between June and December 2 yr after the study lactation for 0.20% of lactations in 2010 and 0.18% of lactations in 2012. The proportion of cows calving 3 yr or more after the study lactation was 0.16% in 2010 and 0.10% in 2011.

When herds that had an autumn-calving component were removed, the proportion of cows that calved again subsequently in the data set was 81% in 2010 and 76% in 2013. Of those cows from spring calving-only herds that calved in 2010, the proportion in calf within 6 wk of MSD_{cow} was 50% in 2010 and 52% in 2013.

Across all years, the majority of the herds had only 1 calving period. The proportion of herds with 1 or more calvings occurring in the autumn months (September to December inclusive) was 43% in 2010 and 38% in 2013.

In all models, milk production and constituents were measured in general earlier than the time period when hazard of pregnancy was assessed; that is, early-lactation milk recording data were used as the exposure

variable on hazard of pregnancy at the following breeding period. Figure 1 shows the distribution of MSD_{cow} to conception intervals of the final enrolled population of cows. In models 1 to 4, the size of the effect refers to the hazard ratio.

Models 1 to 3

The outputs from the multivariable models 1 to 3 are shown in Table 1. The outputs when applied to spring

calving-only herds are shown in Supplemental Table S1 (<https://doi.org/10.3168/jds.2018-15490>). Across all 3 models, hazard of pregnancy was positively associated with fertility and calving subindices. The magnitude of the association was similar between models. Management subindex was negatively associated with hazard of pregnancy across all 3 models.

The PTA for milk yield (kg) was positively associated with the hazard of pregnancy across models 1 to 3 to a similar magnitude. The PTA for fat yield (kg) was

Figure 1. Histogram of days from cow-specific mating start date (MSD_{cow}) to conception for all cows that conceived within the observation period for the final analysis.

Table 1. Outputs from 3 Cox proportional hazards survival models (models 1–3) assessing the associations between milk recording data at 3 lactation stages and hazard of pregnancy

Variable ¹	Model 1 (0–30 DIM)			Model 2 (31–60 DIM)			Model 3 (61–90 DIM)		
	Hazard ratio ²	95% CI	P-value	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Fertility subindex	1.009	1.009–1.009	<0.001	1.009	1.009–1.009	<0.001	1.009	1.008–1.009	<0.001
Calving subindex	1.002	1.001–1.003	0.001	1.002	1.001–1.003	0.001	1.002	1.001–1.003	0.000
Management subindex	0.989	0.987–0.992	<0.001	0.989	0.986–0.991	<0.001	0.989	0.987–0.991	<0.001
Milk (kg) PTA	1.003	1.002–1.004	<0.001	1.003	1.002–1.004	<0.001	1.003	1.002–1.004	<0.001
Protein (%) PTA	1.833	1.510–2.224	<0.001	1.585	1.291–2.945	<0.001	1.403	1.385–1.734	0.002
Fat (kg) PTA	0.934	0.918–0.950	<0.001	0.936	0.920–0.952	<0.001	0.937	0.920–0.953	<0.001
Fat (kg) PTA × fat (kg) PTA	1.000	1.000–1.000	0.007	1.000	1.000–1.000	0.010	1.000	1.000–1.000	0.010
Fat (%) PTA	25.432	10.331–62.603	<0.001	21.607	8.756–53.135	<0.001	22.399	9.115–55.040	<0.001
DIM at test day	0.996	0.994–0.998	<0.001	0.999	0.998–1.001	0.071	1.001	1–1.002	0.160
Yield (kg) at test day	1.010	1.008–1.012	<0.001	1.010	1.009–1.012	<0.001	1.014	1.012–1.016	<0.001
Log-transformed SCC	0.966	0.959–0.974	<0.001	0.948	0.941–0.956	<0.001	0.932	0.924–0.941	<0.001
Lactose (%)	1.398	1.317–1.465	<0.001	1.164	1.101–1.231	<0.001	1.078	1.020–1.139	0.008
Protein (%)	1.123	1.085–1.163	<0.001	1.191	1.142–1.242	<0.001	1.228	1.175–1.283	<0.001
Fat (%)	1.244	1.130–1.369	<0.001	1.050	1.033–1.067	<0.001			
Fat (%) × fat (%)	1.128	1.070–1.190	<0.001						
Parity 1									
Parity 2	0.896	0.873–0.920	<0.001	0.894	0.871–0.918	<0.001	0.872	0.849–0.895	<0.001
Parity 3	0.828	0.803–0.920	<0.001	0.821	0.796–0.847	<0.001	0.797	0.773–0.822	<0.001
Parity 4	0.736	0.712–0.762	<0.001	0.730	0.705–0.756	<0.001	0.707	0.684–0.732	<0.001
Parity 5+	0.511	0.494–0.528	<0.001	0.505	0.488–0.522	<0.001	0.490	0.474–0.506	<0.001
DIM at MSD	1.206	1.193–1.219	<0.001	1.018	1.016–1.021	<0.001	1.015	1.012–1.017	<0.001
DIM at MSD × DIM at MSD	0.965	0.957–0.972	<0.001	1.000	1.000–1.000	<0.001	1.000	1.000–1.000	<0.001

¹MSD = mating start date.²Estimated ratio of the hazards of pregnancy for each unit increase in the exposure variable for all variables except parity, which refers to hazard ratio for each parity category relative to the reference category, which is parity 1.

significantly associated with hazard of pregnancy in all models to a similar magnitude, and in all cases the effect was quadratic. Plotting the coefficients over the range of this variable indicates that there was approximately a negative linear relationship over the range relevant for this population. The PTA for fat concentration (%) was positively associated with hazard of pregnancy across all 3 models. The PTA for protein concentration (%) was positively associated with hazard of pregnancy in all 3 models. Parity was significant in all models.

Milk yield at test day was positively associated with hazard of pregnancy in all 3 models. Each 1-L increase in yield was associated with a 1 to 1.4% increase in hazard of pregnancy. Days in milk at test day was negatively associated with hazard of pregnancy for models using milk recording data within each of 0 to 30 and 31 to 60 DIM. This finding was potentially due to the linear and quadratic effects of DIM at MSD not fully fitting the data, leaving the possibility for DIM at test day to also have an effect. Log-transformed SCC was negatively associated with subsequent hazard of pregnancy in models 1, 2, and 3, all by a similar magnitude.

Milk lactose concentration was positively associated with later hazard of pregnancy in all models. From 0 to 30 DIM, each 1-percentage unit increase in milk lactose concentration was associated with a 39% increase

in hazard of pregnancy when subsequently bred. The magnitude of this effect estimate was reduced in the window of 31 to 60 DIM, where 1-unit increases were associated with a 16% increase in pregnancy hazard at the ensuing breeding season. It was further decreased in model 3, where a 1-unit increase in milk lactose concentration was associated with a 7% increase in the hazard of pregnancy.

Milk protein concentration was positively associated with the hazard of pregnancy at the following breeding season in all 3 models. For a 1-unit increase in milk protein concentration in models 1 and 2 (0–60 DIM), there was a 12 and 19% increase in the hazard of pregnancy between models 1 and 2, respectively. In model 3 (61–90 DIM), the magnitude of this effect was greater, with a 22% increase in the hazard of pregnancy for a 1-unit increase in milk protein concentration.

Milk fat concentration was associated with hazard of pregnancy in models 1 and 2. The effect was quadratic in model 1 (0–30 DIM), with the linear and quadratic terms inverted. Plotting the coefficients revealed that the relationship was positive up to a maximum of 4.6%, after which it declined, during the first milk recording window. Milk fat concentration was positively associated with fertility performance, with each unit increase in milk fat concentration resulting in an increase in the

Table 2. Output from model 4, a Cox proportional hazards survival model assessing the association between combined milk recording data from 0 to 90 DIM and hazard of pregnancy

Item ¹	Hazard ratio ²	95% CI	P-value
Fertility subindex	1.009	1.008–1.009	<0.001
Calving subindex	1.002	1.001–1.003	<0.001
Management subindex	0.989	0.987–0.992	<0.001
Milk (kg) PTA	1.001	1.000–1.001	<0.001
Protein (%) PTA	1.419	1.151–1.750	<0.001
Fat (kg) PTA	0.994	0.992–0.996	<0.001
Peak yield (kg)	1.069	1.057–1.082	<0.001
Peak yield × peak yield	0.999	0.999–0.999	<0.001
Mean SCC	1.152	1.077–1.231	<0.001
Mean SCC × mean SCC	0.977	0.970–0.984	<0.001
Nadir protein (%)	1.277	1.219–1.340	<0.001
Mean lactose (%)	94.255	6.765–1,313.223	0.001
Mean lactose (%) × mean lactose (%)	0.641	0.487–0.843	0.002
Maximum fat (%)	1.273	1.114–1.454	0.010
Maximum fat (%) × maximum fat (%)	0.974	0.910–0.989	<0.001
Parity 1			
Parity 2	0.864	0.840–0.888	<0.001
Parity 3	0.797	0.771–0.823	<0.001
Parity 4	0.713	0.687–0.739	<0.001
Parity 5+	0.500	0.483–0.518	<0.001
DIM at MSD	1.018	1.016–1.021	<0.001
DIM at MSD × DIM at MSD	1.000	1.000–1.000	<0.001

¹MSD = mating start date.²Estimated ratio of the hazards of pregnancy for each unit increase in the exposure variable for all variables except parity, which refers to hazard ratio for each parity category relative to the reference category, which is parity 1.

hazard of pregnancy by 1.05 when measured at 31 to 60 DIM.

Model 4

The outputs from model 4 are shown in Table 2. The outputs when applied to spring calving-only herds are shown in Supplemental Table S2 (<https://doi.org/10.3168/jds.2018-15490>). Similar to the first 3 models, both the fertility and calving subindices (made up of calving difficulty, gestation length, and calf mortality traits) were positively associated with hazard of pregnancy in model 4. Predicted transmitting ability for both protein concentration and milk yield was positively associated with hazard of pregnancy. Predicted transmitting ability for fat yield was negatively associated with the hazard of pregnancy. Peak yield was significantly associated with hazard of pregnancy, and the relationship was found to be quadratic. Plotting the coefficients suggested that the relationship is positive up to a peak yield of 40 kg. In the final combined model, mean log-transformed SCC was significantly associated with the hazard of pregnancy at subsequent breeding, and the relationship was found to be quadratic. Plotting of the coefficients demonstrated a curvilinear relationship, with the hazard of pregnancy decreasing

approximately linearly after a mean log-transformed SCC of 3.25, with a maximum of 3.0.

For lactose concentration, the best model fit was achieved by including the mean over the 3 recording windows as a linear and second-order polynomial term. For lactose, hazard of pregnancy increased approximately linearly up to 4.5%, after which further increases were associated with only small gains in hazard of pregnancy up to a maximum point at 5.1%. Protein concentration was best included as the minimum value over the 3 windows, with the higher nadir positively associated with hazard of pregnancy. For milk fat, the best summary variable was maximum fat concentration, and again it was best included as a linear and second-order polynomial term. Milk fat concentration was significantly associated with hazard of pregnancy at subsequent breeding when assessed over the 3 milk recording windows. It increased approximately linearly up to 3.8%, after which further increases were associated with small gains in hazard of pregnancy up to a maximum point of 4.6%.

Accelerated Failure Time Models

Table 3 shows the difference in predicted median time from MSD_{cow} to conception for cows in the 10th

percentile versus those in the 90th percentile for each variable. Similarly, Supplemental Table S3 (<https://doi.org/10.3168/jds.2018-15490>) shows predicted proportions of animals not in calf at 42 and 84 days, for cows at the 10th and 90th percentile for each variable. The predicted survival curves for some of the main findings are shown in Figure 2. In general, the effect of milk constituents is relatively modest, with differences in median time to conception of 0 to 10 d, compared with the effect of the fertility subindex, which had a predicted difference of 62 d (Figure 2), between cows at the 10th and 90th percentiles for the variable of interest. Of the primary milk constituents measured, milk lactose concentration had the greatest effect on fertility performance at the following breeding period, particularly when measured in the first 30 DIM (Figure 2), when comparing cows at the 10th and 90th percentiles. Milk protein concentration had the greatest effect in model 4 (i.e., when modeled as the mean nadir over the 3 recording windows), with a 7-d increase in time from MSD_{cow} to conception between animals in the 10th and 90th percentiles. Of the first 3 models, milk protein concentration had the greatest effect in the 61 to 90 DIM recording window, with a 9-d increase in time from MSD_{cow} to conception between animals in the 10th and 90th percentiles (Figure 2). Finally, milk fat concentration was significant in models 1 and 2; however, this variable only gave rise to 2- and 3-d increases in median time from MSD_{cow} to conception in models 1 and 2, respectively, when cows in the 10th and 90th percentiles were compared. The mean of log-transformed SCC across the 3 recording windows was significantly negatively associated with MSD_{cow} to conception interval. This resulted in a 7-d difference in time to conception for cows in 10th versus 90th percentiles of mean log-transformed SCC (Figure 2).

DISCUSSION

This study identified significant associations between early-lactation milk composition and subsequent fertility performance during the breeding season in Irish milk-recorded, spring-calving cows in this study population. The final models were also applied to a subset of data from herds with only a spring-calving component (Supplemental Tables S1 and S2; <https://doi.org/10.3168/jds.2018-15490>). Although the herd-level performance of spring calving-only herds was greater than that of the overall data set in terms of the proportion in calf within 6 wk of MSD_{cow}, there was very little effect on the effect estimates at the individual-cow level.

Across all 4 models, fertility subindex was positively associated with hazard of pregnancy and had a large effect on subsequent hazard of pregnancy (Figure 2). The

findings from this study population differ with those of Morton et al. (2017), who found that increasing breeding value for fertility from low to high had a smaller effect than increasing milk protein concentration from low to high on the fertility measure used. These differences could be due to differences in the calculation of breeding value estimates or in the sire selections used in Ireland compared with Australia. The large effect of the genetic variables observed in our study in cows with low genetic merit would be less in populations with less variation in the genetic variables between cows.

Our results are similar to other Irish studies in which cows with superior genetic values for fertility had greater probability of conception (Moore et al., 2014; Fenlon et al., 2017). The EBI used in Ireland is updated several

Table 3. Differences in median cow-specific mating start date to conception time between the 10th and 90th percentiles for each variable

Item	Median difference ¹ (d)
Common variables ²	
Fertility subindex	62
Calving subindex	6
Management subindex	−6
Protein (%) PTA	5
Fat (%) PTA	46
Milk (kg) PTA	66
Fat (kg) PTA	−52
Parity	−53
Model 1	
Lactose (%) ³	10
Log-transformed SCC	−5
Protein (%)	5
Milk yield at test day (kg)	9
Fat (%)	2
DIM at test day	−5
Model 2	
Lactose (%)	3
Log-transformed SCC	−7
Protein (%)	7
Milk yield at test day (kg)	7
Fat (%)	3
DIM at test day	−2
Model 3	
Lactose (%)	1
Log-transformed SCC	−10
Protein (%)	9
Milk yield at test day (kg)	9
DIM at test day	−1
Model 4	
Mean SCC	−7
Peak yield (kg)	7
Nadir protein (%)	7
Mean lactose (%)	4
Maximum fat (%)	0

¹Difference in predicted median times from cow-specific mating start date to conception between cows in the 10th percentile and those in the 90th percentile for each variable, with the remainder of the variables equal to the median of the population. Days are rounded to the nearest integer.

²Variables common to all models, predicted from model 1.

³Parity 1 compared with parity 5+.

Figure 2. Predicted survival curves for cows at the 10th and 90th percentiles for the variable of interest. All other variables were kept constant equal to the median of the population. (A) Predicted survival curves for cows with fertility subindices (Fert SI) of -25.0 (10th percentile) and 105.9 (90th percentile). (B) Predicted survival curves for cows with milk lactose percentage of 4.48% (10th percentile) and 4.99% (90th percentile). (C) Predicted survival curves for cows with milk protein percentage of 2.84% (10th percentile) and 3.49% (90th percentile) for cows 61 to 90 DIM. (D) Predicted survival curves for cows with log-transformed SCC (lnSCC) of 3.13 (10th percentile) and 5.79 (90th percentile) averaged over the first 3 milk recordings.

times per year using daughter performance data for all sires. Breeding values are composed mostly from the genetic values of the cow's sire and maternal grandsire. Because the animal's own performance will be one of many individuals contributing to her sire and maternal grandsire's breeding values, her own performance will have contributed to her own breeding value. However, because EBV comprises mainly the genetic values of the cow's sire and maternal grandsire, which are based on data from a large number of progeny, and because of the low heritability of the genetic measures used, the individual's contribution to her own breeding value is close to zero. To investigate this, we also fitted the final model to subsets of cows whose sires had more than 100, 1,000, and 10,000 daughters. The effect estimate did not change across these data sets.

A positive relationship between PTA for milk protein concentration and hazard of pregnancy was evident in models 1 to 4, indicating that cows with superior genetic merit for milk protein concentration are also likely to be cows with better fertility performance. This finding is similar to other studies in which a positive association between milk protein and fertility was reported in early lactation (Morton et al., 2017). That same study outlines that the relationship between milk protein concentration and fertility performance has both genetic and nongenetic components. In a study by Morton et al. (2018), substantial genetic covariance between milk protein concentration up to 120 DIM and fertility was found.

Milk yield at test date was positively associated with hazard of pregnancy in all 3 models. A 1 to 1.4 percentage unit increase in the hazard of pregnancy resulted for each 1-kg increase in yield at test day between 0 and 90 DIM. In model 4, peak yield was also positively associated with hazard of pregnancy, at least up to 40 kg. Cook and Green (2016) found a positive association with probability of conception by 100 and 150 DIM with average daily milk yield in wk 4 of lactation. Similarly, Buckley et al. (2003) found a positive relationship between milk yield at first service and the likelihood of being pregnant in the first 6 wk of the breeding season. This finding could be related to better control of negative energy balance, resulting in better fertility performance. It has been suggested that improvements in fertility performance observed with increased milk yield could reflect better-managed herds and overall improved health (Buckley et al., 2003; Bello et al., 2012).

The effect of the association of SCC, measured as log-transformed SCC, on hazard of pregnancy was identified as a negative association in all models. The difference between the 10th and 90th percentiles of mean log-transformed SCC (across 0–90 DIM) was 7 d

in MSD_{cow} to conception (Figure 2). It has been shown that both clinical and subclinical mastitis can detrimentally affect fertility (Hudson et al., 2012).

Milk lactose concentration was found to have a positive association with hazard of pregnancy in all models. There was a 10-d difference in MSD_{cow} to conception interval between the 10th and 90th percentiles of milk lactose concentration at first test day (Figure 2). Madouasse et al. (2010) found that higher percentage of lactose on the first test day was associated with a higher probability of conception by 145 DIM. Higher early-lactation milk lactose content has been linked to earlier resumption of ovarian activity as milk lactose is derived from glucose supplied to the mammary gland and may serve as a proxy measure for energy balance (Reksen et al., 2002). Factors that cause milk lactose concentration, particularly at 0 to 30 DIM, appear to be a useful predictor of subsequent fertility in spring-calving cows and may be related to energy status.

The greatest effect estimate of the association between test day milk protein concentration and hazard of pregnancy was identified in model 3 (61–90 DIM). This may suggest an association of factors affecting milk protein concentration also affecting fertility performance when measured when cows should be close to peak yield. Although a positive relationship between milk protein concentration and hazard of pregnancy was identified across models 1 to 3 in terms of reproductive performance, differences of only 6 to 9 d were identified when comparing the cows in the 90th percentile with those in the 10th percentile for milk protein concentration. The association between milk protein and fertility performance is reflected in other studies. Morton et al. (2016) showed a positive relationship between early-lactation milk protein concentration and fertility performance, accounting for genetic values. The same study also showed a difference in this effect across different yields, with a weaker effect seen at higher early-lactation milk yields. Morton et al. (2017) reported that both milk protein concentration and Australian fertility breeding value were associated with performance measures used to assess fertility but were independent and additive. Cook and Green (2016) demonstrated an increased probability of conception by 100 and 150 DIM with increased milk protein concentration at both first and second test days. In model 4, the nadir milk protein concentration was positively associated with hazard of pregnancy. Therefore, the higher the value of the lowest milk protein concentration (demonstrated by the lactation curve at peak yield), the greater the hazard of pregnancy. Similarly, Buckley et al. (2003) demonstrated that cows reaching nadir milk protein concentration later in lactation had reduced pregnancy to first service.

Milk fat concentration was positively associated with hazard of pregnancy in model 1 (0–30 DIM); however, the relationship was curvilinear and was only present up to a maximum of 4.6%. In model 4, the difference between the 10th and 90th percentiles was 0 d in median time to conception. Other studies have identified higher fat concentrations in early lactation (<30 DIM) as negatively associated with subsequent fertility (Madouasse et al., 2010; Cook and Green, 2016). Higher milk fat percentages were found to be negatively correlated with energy balance (Grieve et al., 1986) and with health and fertility (Heuer et al., 1999).

Survival analysis is a useful tool for studying fertility. Hazard ratios from Cox proportional hazard models estimate strength of association (i.e., the expected effect of a 1-unit increase in the exposure variable). In contrast, we estimated the effect of each variable in cows with low values for the exposure variables using parametric accelerated failure time models to predict median survival times for otherwise identical animals at the 10th and 90th percentiles of the variable of interest. Thus, effects estimated using our method depend jointly on the strength of association and the amount of variation in the exposure variable in the study population. An exposure variable that is strongly associated with the dependent variable will have little effect in cows with low values for the exposure variable if there is little variation in that exposure variable.

This method provides a useful comparison of the likely fertility performance of cows at the extremes of the distribution for a given variable. However, it is important to note that the magnitude of this difference will be much less if it is applied to the overall population (i.e., if each animal in the population were replaced with an otherwise identical cow but at the 90th percentile for the variable of interest). This is demonstrated in Supplemental Table S4 (<https://doi.org/10.3168/jds.2018-15490>), which shows the effect of replacing each cow in the population with an otherwise identical cow but at the 90th percentile for the variable of interest. Although the order of the variables in terms of relative effect is not affected, the magnitude of the effect is lower than the comparison between cows and the 10th and 90th percentiles. The magnitude is lower because the difference in days open is relative to a baseline of the median predicted survival times across all of the cows in the population rather than relative to a cow at the 10th percentile for the variable of interest. The practical usefulness of altering these variables to improve fertility performance should take into account the fact that the difficulty and cost of improving each variable will differ and that our findings imply only association and may not show a direct causal link.

We used MSD_{cow} to conception interval as the outcome measure based on individual milk recording data, similar to other studies (Madouasse et al., 2010). In the absence of fertility data, we created proxy measures based on milk recording cow-level data. It is difficult to compare results with the results of other studies due to heterogeneity in the fertility outcome used as well as the differing measures of milk composition. Interestingly, in a recent UK-based study, Hudson and Green (2018) concluded that the use of milk recording data was limited in predicting herd-level conception risk. As fertility performance is usually assessed at the herd level, to make more meaningful conclusions using our data, we would need to assess the outcomes at the herd level.

The unit of measure of reproductive risk in this study used milk recording data only to generate the dependent variable, MSD_{cow} to conception interval. This is not a frequently used method and, unlike the work of Morton et al. (2016), has some limitations with regard to missing information about the cows that failed to conceive. Several weaknesses of this approach need to be considered. We used subsequent calving dates to approximate conception dates in each lactation using a fixed gestation length. In reality, gestation lengths vary on either side of the fixed length used, leading to errors in the actual conception date that is approximated. However, these errors are likely to be randomly distributed around zero and with a large data set are not likely to adversely affect the results. We discarded milk records from cow lactations with a calving to conception date of <20 d; however, this consisted of only 0.4% of milk records.

The data were extracted from a single source, with no availability of culling data, although all animals in the data set were milk recorded and therefore present on the farm at 3 mo of lactation. Because we used a subsequent calving date to determine conception dates, it is possible that some animals did in fact conceive but had no recorded subsequent calving because they were culled from the herd. These animals would have been incorrectly assumed to have not had a conception date and consequently right censored. However, it is expected that this would have affected only a small proportion of cases given that the primary reason for culling in a seasonal system is infertility (Rajala-Schultz and Gröhn, 1999).

Conventionally a form of voluntary waiting period was used to determine when cows are eligible for service. In our study, we have used MCII, which more accurately considers when a cow is eligible for conception and specifically refers to when a cow that calves after or close to the MSD_{herd} may be considered at risk

of conception. Cows will not conceive in the immediate postpartum period (Crowe, 2008). Between 20 and 42 d postpartum, the conception rate is likely to be low. However, several factors will influence the cost benefit of inseminating cows during this period. In practice, the decision to serve cows in this window varies between farms and between cows on the same farm. We enforced a farm year-specific MCII that equated to the earliest DIM (>20) that an animal conceived in that herd in that year, up to a maximum of 42 DIM, to account for when cows were at a biologically plausible risk of conception. We used estimated conception dates to define herd breeding windows, resulting in errors setting the MSD. However, these would have been accounted for to some degree by including herd as a frailty effect in the analysis.

CONCLUSIONS

Our study identified several associations between readily available test day variables and days open, measured as MSD_{cow} to conception interval, in the study population of Irish spring-calving, milk-recorded cows that are consistent with previous literature. The nature of the data set used and how the measures of fertility were calculated using only milk recording data are important in terms of limiting comparisons with other studies. However, our analysis showed that although the associations between milk constituents and fertility were statistically significant, their overall effect in determining pregnancy outcomes between cows at extremes of the population (i.e., the 10th and 90th percentiles) for that variable are relatively modest, particularly when compared with fertility genetic sub-index. Of the constituents measured, milk lactose concentration measured at 0 to 30 DIM had the greatest effect in determining fertility outcome at subsequent breeding, with a 10-d increase in predicted median time to conception in the 10th percentile compared with the 90th percentile. The predictive value of test day milk composition data on hazard of pregnancy within a spring-calving context appears to be relatively low to moderate at the individual-cow level. Further work is required to test the usefulness of these associations at the herd level.

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