RISK FACTORS FOR EARLY PREGNANCY LOSS IN PASTURE-BASED DAIRY COWS

RW Shephard *Herd Health Pty Ltd, 65 Beet Road, Maffra, VIC, 3860, Australia*
JM Morton *Jemora Pty Ltd, Geelong, VIC, 3220, Australia*
L Reynolds *ACE Farming Company, Sydney, NSW, 2000, Australia*

Abstract

Cows from 4 pasture-based dairy herds diagnosed pregnant by transrectal ultrasound pregnancy diagnosis at day 35 of pregnancy were observed for pregnancy loss. Serial ultrasound pregnancy diagnosis tests were repeated twice at around 60-day intervals. Pregnancy loss was defined as a previously identified pregnancy confirmed lost or a cow confirmed pregnant but to a later conception date at subsequent examination. Hypothesized interrelationships between cow age, breed, milk production and milk composition at peak lactation, body condition score at start of the mating period, estimated genetic breeding value for fertility, number of days calved at conception, whether the pregnancy was to a fixed-time (synchronized) artificial insemination, occurrence of clinical mastitis, and pregnancy loss were described a priori using a causal diagram. Associations were examined using Cox proportional hazards regression models for interval-censored data with covariates selected using the causal diagram. Ninety pregnancies were lost from the 45th day of pregnancy from 1,149 pregnancies (7.8%). Risk of pregnancy loss increased as peak milk production deviated from 30 litres per day (P=0.005), and in cows experiencing clinical mastitis after first positive pregnancy diagnosis (2.7-fold increased hazard; P=0.03). There was a trend towards increased pregnancy loss in cows with peak lactation milk fat concentrations well below or well above 4.1% (P=0.07). Incidence of pregnancy loss in grazing dairy cows may be reduced if joint causes of low peak daily litres and pregnancy loss are removed (including implementation of strategies to better manage cows producing high peak daily litres), and clinical mastitis incidence is reduced.

Introduction

Dairy cows that lose their pregnancy after their first positive pregnancy diagnosis reduce herd profitability. In seasonal and split calving herds, most such cows are non-pregnant at the end of the mating period and are either culled at the end of lactation or retained ('carried over') and at reinseminated at the next mating period in the herd. Dairy farmers and veterinarians have reported that the proportion of dairy cows diagnosed pregnant in their first trimester that subsequently fail to calve or that calve to an apparently later conception date is increasing. A review of pregnancy loss identified losses in the critical third period of pregnancy (28–60 days) as strongly influencing herd reproductive efficiency, and very low cow survival in cows that lose a pregnancy within this period[8]. These cows also complicate herd management. Early pregnancy testing (5–13 weeks post insemination) is a recommended management practice for Australian dairy herds[1.] However, the risk of pregnancy loss is greater in early pregnancy[2] and so follow-up testing is often required to identify cows that subsequently lose their pregnancy after their first positive pregnancy diagnosis.

The incidences of embryonic losses between days 7 and 16 of pregnancy have recently been estimated at 26% in heifers and 34% in multiparous cows3. Between 7–8% of pregnancies are estimated to be lost between days 30 and 90 of gestation in high-producing cows, with most losses occurring before day 434. In pasture-based cows in New Zealand, incidences were 2.9% between approximately days 28 to 70 of pregnancy and 0.7% between approximately days 70 to 98 of pregnancy5. Incidences between days 31 and 45 of pregnancy in high-producing Californian Holstein cows were 12.5% (range between herds: 7.3 to 15.3%)6 and 11.4% between days 35 and 63 of gestation in another North American study[7].

The variation in reported incidences of pregnancy loss after first positive diagnosis between countries and production systems means local studies are needed to estimate the incidence of pregnancy loss in pasture-based Australian dairy herds. There is also a need to identify risk factors for pregnancy loss in Australian dairy cows, to allow preventive strategies to be developed. An observational study to address these aims was conducted in 2015–2016 in 4 herds in Victoria, Australia.

Materials and methods

Four pasture-based dairy herds from a corporate farming enterprise located in the Macalister Irrigation District of Victoria, Australia, were selected for the study by convenience sampling. These herds provided suitable production, ancestry, mating and mastitis records. Each herd employed two distinct compact calving periods in a year (split-calving). This was achieved through the use of two restricted mating periods. Split-calving is commonly-used in Australian dairy herds. In a recent study, split-calving was used in 37% of Australian dairy herds, with seasonal calving (a single compact calving period) used in 40% and year-round calving used in 23%9. The spring-calving and autumn-calving groups were approximately equal sized within each of the four herds. Cows for this study were selected from those that were inseminated in the 2015 spring mating period in each herd.

Whole herd oestrus synchrony with fixed time artificial insemination

(FTAI) was used on the first day of the spring mating period (day 1) in each herd. After FTAI, cows were fitted with heat mount detectors after FTAI and daily oestrus detection with AI of cows detected in oestrus occurred over the next 10–11 weeks. Each herd used pregnancy diagnoses on three occassions to identify pregnancies to artificial inseminations (AI) early in the mating period (performed during the mating period), pregnancies to subsequent matings (performed after the end of the mating period) and to confirm pregnancy retention in cows previously diagnosed pregnant (performed just prior to drying-off). Thus, pregnancy testing was timed to occur at approximately 60-day intervals starting from around 35 days after the start of the mating period (the day of FTAI) until 179, 181, 180 and 169 days after the start of the mating period for each herd, respectively. All cows were to be examined for pregnancy on each occasion. A single experienced veterinarian performed all ultrasound pregnancy testing.

Only cows that were confirmed pregnant at either of the first two of these pregnancy tests and that had at least one further pregnancy test after the initial positive diagnosis were enrolled in the study. All study cows therefore provided a period of pregnancy observation after first confirmation of pregnancy. Pregnancy loss was defined as occurring when a cow with a positive pregnancy test had a subsequent negative pregnancy test or whose later positive pregnancy test provided an estimated conception date that was 21 days or more after the original estimated conception date. The late embryo period has been defined as between 21–42 days of gestation, followed by the fetal period from day 43 of gestation to term5. Because pregnancies could have been monitored for losses during both the embryonic and fetal periods, the term 'pregnancy loss' is used in this paper to describe any losses.

All cows were individually identified using ear tags with these numbers linked to their unique national dairy cow identification number. An experienced veterinarian measured cow body condition score on a 1–8 scale, where 1 indicates emaciated and 8 indicates extremely fat[10]. Body condition scoring was performed during milking on rotary dairy platforms around the first day of each herd's mating period. Cow breed, date of birth, sire and dam identification, sire and dam estimated genetic breeding values for fertility (Australian Breeding Values for daughter fertility, calculated in February 2017) and calving dates were obtained from MISTRO Farm™ herd management records and the national database data via each cow's national cow identification number11. The Australian Breeding Value for daughter fertility is an estimate of the genetic merit of an individual for producing daughters with high reproductive performance. Daily milk production (litres), milk composition (fat concentration and protein concentration) were recorded for two consecutive milkings combined for each cow at monthly intervals conducted across the study period by professional herd testers from HiCo Co-operative Australia Limited, a commercial milk recording organization. Individual cow clinical mastitis events as detected at milking by herd staff were recorded.

The unit of analysis was the cow pregnancy. No cow contributed more than one pregnancy to the study, so this unit of interest was equivalent to the cow and the term 'cow' is used for ease of description.

Cow ages in years at calving were calculated as ((calving date minus date of first calving) divided by 365) plus two and rounded to the nearest year. Breeds were supplied as 4-character breed codes where the 1st, 2nd, 3rd and 4th characters reflected the breeds of the cow's paternal grand sire, paternal grand dam, maternal grand sire, and maternal grand dam, respectively. Using these, cows were classified as Holstein-Friesian (all four grandparents designated Holstein-Friesian), Jersey (all four grandparents designated Jersey) or crossbreed (at least two different grandparent breeds designated). For remaining cows, breed code was not available or one or more characters in the breed code were undefined (i.e. 'X'). Australian Breeding Values for daughter fertility were estimated for each cow as sire Australian Breeding Value x 0.5 + dam Australian Breeding Value x 0.5, or where there was no dam information as sire Australian Breeding Value x 0.5 + 50 (i.e. the cow's dam's Australian Breeding Value was assumed to be the breed average value of 100). Calving to conception intervals were categorized into three groups: 1-60 days (representing late spring-calved cows), 61-120 days (early spring-calved cows) and more than 120 days (predominantly 'carryover cows' — cows that did not calve during the most recent spring calving period but calved in a previous calving period).

The first milk recording after the start of the mating period was used to define peak daily milk production of each cow. Milk production values at that first milk recording event (litres, fat and protein yields) were adjusted to account for differences in stage of lactation between cows at that milk recording to provide peak daily estimates. To do this, milk recording data from 200 herds were obtained from the local herd-testing centre, and daily production per cow for each of litres and kilograms of fat and protein for the cow at the milk recording regressed on stage of lactation at that milk recording (described using month number integer) using polynomial (cubic) regression. Ratios between the highest fitted daily production per cow and fitted daily production values for each month of lactation were then calculated. Peak lactation daily milk production of liters, kilograms of fat and protein were estimated for each cow. This was achieved by adjusting each cow's daily production as estimated at her first herd test after calving using the regression equation and her month of lactation at first test described previously. These estimates at peak lactation were used in all statistical models to control for differences in stage of lactation at first herd test between cows.

Raw data was aggregated into comma separated value tables and imported into the R Language and Environment for Statistical Computing V3.2 for cleaning, manipulation and analysis[12]. Descriptive statistics describing pregnancy loss proportions within subsets of the cow population were calculated. Exact binomial confidence intervals for pregnancy loss proportions were calculated without accounting for clustering by herd. The exact day of pregnancy loss cannot be determined from sequential pregnancy testing conducted at long intervals. Pregnancy loss could occur at any time in the period from the day after the first positive pregnancy test to the day when the pregnancy loss was identified. Thus, when calculating times from first positive pregnancy test to pregnancy loss, these were interval-censored survival data. Accordingly, pregnancy loss was modelled using a modification of the Cox proportional hazards regression analysis model, modified to account for interval-censored data. The R library icenReg 1.3.6 was used.

A causal diagram describing hypothesized and plausible causal (and confounding) pathways between explanatory variables, and between these and the binary outcome variable (pregnancy loss) was developed (Figure 1) using the R library dagitty 0.2.213. Body condition score was assessed only once for each cow, around the first day of each herd's mating period. This was after peak lactation for most cows. However, body condition at the start of the mating period

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is likely to be positively correlated with body condition at calving so our body condition score data were considered to be a surrogate for body condition at calving, and this was reflected in the pathways from body condition score included in the causal diagram.

The total effects of each explanatory variable (i.e. the combined effects of all hypothesized pathways from the explanatory variable to pregnancy loss) were estimated with separate models for each explanatory variable, with all potential confounding variables fitted as covariates14. Any variable with separate (direct or indirect) pathways to each of the explanatory variable and pregnancy loss in the causal diagram was a potential confounding variable.

The Australian Breeding Values for daughter fertility were not comparable between breeds and these data were not available for many cows. Therefore, this variable was not included as a covariate in any models despite being required for some models based on the causal diagram. Instead, the subset of data containing only Holstein-Friesian cows was used to examine the effect of estimated Australian Breeding Value for daughter fertility on pregnancy loss. This was done to examine if part of the mechanism for superior daughter fertility in individuals of high genetic merit for fertility is mediated, in part, though reduced risk of pregnancy loss.

Herd was forced into all models to account for clustering of the dependent variable within herd, and to adjust for confounding by herd. Numbers of cows within each level of categorical variables were examined. Where necessary, categories were aggregated to provide sufficient observations for meaningful analysis. The effect of clinical mastitis was assessed after classifying each cow as either having one or more cases of clinical mastitis or no cases between its first positive pregnancy diagnosis and its final pregnancy test or, for cows losing their pregnancy, the first pregnancy test where the pregnancy loss was identified. Additional analyses were performed to further explore the effects of peak production. To help identify any curvilinear relationship with continuous exposure variables, values were grand mean centered, those transformed data squared, and these linear and quadratic terms were simultaneously fitted into statistical models.

A high proportion of cows had missing data for date of birth and breed. This was primarily because of expansions occurring within the study herds through purchase of many cows at herd dispersal sales. Many of these purchased cows had incomplete data transfer of their records to the new herd. For this reason, date of first recorded calving obtained from national records was used to estimate ages of cows whose date of birth was not available. The year of birth was assumed to be 2 years before the cow's first recorded calving. This resulted in only one cow without an age at calving estimate. Complete breed records could not be obtained for 624 of the 1,149 cows in the study. Two analyses were therefore undertaken for models in which breed was a potential confounder (from the causal path diagram). One model included breed (so fewer cows were included) and breed was not fitted in the other model to increase precision of the estimated coefficient for the explanatory variable of interest. Coefficient estimates were compared between the two models to assess the impact of any confounding by breed in the breed-excluded models.

The significance of relationships between proposed explanatory variables and pregnancy loss was assessed using likelihood ratio test p-values.

Results

A total of 1,756 cows were to be inseminated during the spring 2015 mating period in each herd (452, 322, 330 and 652 in herds 1 to 4, respectively). Of these, 1,217 were diagnosed as having become pregnant during that mating period (305, 247, 246 and 419 in herds 1 to 4, respectively). Of these, 1,149 pregnant cows had at least one subsequent pregnancy test (302, 210, 237 and 400, in herds 1 to 4, respectively) after their first positive diagnosis and were enrolled into the study. These distribution of cows' most recent calvings were: autumn 2014 (1 cow); spring 2014 (47 cows); autumn 2015 (298 cows); spring 2015 (802 cows) and unknown (1 cow). Cows that were more than 120 days calved by the start of the spring mating period (carryover cows) were slightly older (5.6 years versus 5.1 years), were in slightly heavier body condition score (median BCS 4.75 versus 4.50), had higher peak lactation litres (33.5 versus 28.4), lower peak lactation milk fat percentage (4.0 versus 4.2) and similar peak lactation milk protein percentage (3.2) than cows that were less than 120 days calved by the start of spring mating. The first spring herd test of each herd returned bulk milk cell counts of 209, 164, 131 and 181 thousand cells per mL of milk for herds 1, 2, 3 and 4, respectively.

The distributions of cows, and numbers and proportions experiencing pregnancy loss for each category of each potential risk factor are presented in Table 1. A total of 90 pregnancy losses were observed from the 1,149 pregnancies or 7.8% (95% CI 6.3% to 9.5%; range between herds 5.2% to 10.9%). The median stage of pregnancy at first positive pregnancy test was 45 days (mean 41.8 days; range 22 to 148 days; 25th and 75th percentiles 35 and 54 days, respectively), and the median interval from first diagnosis of pregnancy to final pregnancy test was 99 days (mean 112.9 days; range 3 to 149 days; 25th and 75th percentiles 96 and 145 days). Final pregnancy test was a median of 161 days (mean 154.7 days; range 33 to 209 days; 25th and 75th percentiles 136 and 180 days) after conception date. No cow identified to have lost a pregnancy was subsequently confirmed pregnant again across the remainder of the study period. These losses occurred between days 35 and 161 after conception.

A total of 7, 34, 0, and 24 cases of clinical mastitis were recorded immediately preceding or during the spring mating period for herds 1, 2, 3 and 4, respectively. This provided a total of 67 cases and a cumulative incidence of 5.8% of cows (1.6%, 10.6%, 0.0%, and 0.2% in herds 1, 2, 3 and 4, respectively). Of these 67 cases, 43 were identified as occurring after conception in cows with a confirmed pregnancy.

Hazard ratios and 95% confidence intervals for the estimated total effects of explanatory variables are presented in Table 2 (continuous variables) and Table 3 (categorical variables). Survival curves for pregnancy retention derived from the individual hazards models are presented in Figure 2 for peak daily litres per cow and Figure 3 for clinical mastitis. Peak daily litres per cow and clinical mastitis were significant predictors of pregnancy loss. For peak daily milk litres per cow, inclusion of the quadratic term improved fit of the model over just the linear term (Table 2; P=0.007 for assessing the null hypothesis that the quadratic term coefficient is 0 when the linear term is already included in the model) such that cows producing less than or more than 30 litres per day at peak lactation had greater risk of pregnancy loss compared to cows peaking at 30 litres. Risk was greater the further peak production was from 30 litres in both directions. This was reflected in the survival curves for both 20 and 40 litres per cow declining more rapidly than for 30 litres (Figure 2). The rapidity of decline in survival curves was greater for 40 litres than for 20 litres, indicates that the relationship is asymmetric, with cow pregnancy loss risk increasing more for every additional litre of milk above a 30-litre lactation peak than for every additional litre of peak milk below a 30-litre lactation peak. Cows experiencing clinical mastitis after their first positive pregnancy diagnosis had increased risk of pregnancy loss compared to cows not affected by clinical mastitis in that period. The survival curves for pregnancy retention reflected this with more rapid decline for cows that experienced clinical mastitis (Figure 3).

The effect of peak daily litres was further modelled to explore relationships with other variables. Simultaneous inclusion of milk protein concentration or milk fat concentration at peak lactation (linear term only) did not improve model fit (P=0.51 and P=0.93, respectively), and there was no significant interaction between peak daily litres (each of linear and quadratic terms) and herd (P for interaction terms collectively 0.34). The potential confounding effect of cow breed on the effect of peak daily litres, and possible interactions between peak daily litres and breed, on pregnancy loss risk were assessed by fitting a separate model examining the effect of peak daily litres using only Holstein-Friesian cows (357 cows). This model returned very similar coefficients for peak daily litres to when cows of all breeds were used, and breed was not fitted as a covariate (hazard ratios for linear and quadratic terms were 1.024 and 1.002, respectively, compared to 1.013 and 1.003, respectively, when cows of all breeds were used). Thus, estimates when cows of all breeds were used, and breed was not fitted as a covariate were similar to those from just Holstein-Friesian cows.

To further understand the relationship between peak daily litres and pregnancy loss, effects of daily total solids (fat plus protein kilograms) per cow and milk fat concentration, both at peak lactation, were also assessed. The effect of daily total solids on pregnancy loss risk was estimated adjusted for cow breed, age, body condition score and herd. Total solids (without litres) (linear term only) was not a significant predictor of pregnancy loss although the hazard ratio point estimate was consistent with increased solids production being associated with a higher risk of pregnancy loss (hazard ratio for a 1 kg increase in peak daily total solids per cow: 1.61, 95% CI 0.91-2.83, P=0.072). There was close correlation between daily litres and total solids production at peak lactation (Pearson's r=0.87) but the relationship was also heteroscedastic. Covariance increased as production increased suggesting greater variation in protein and fat concentrations amongst high-producing cows than amongst low-producing cows. There was also some evidence of effects of milk fat concentration at peak milk production on pregnancy loss risk (joint P for linear and quadratic terms 0.07). Based on those estimates, cows were at increased risk of pregnancy loss when milk fat concentration at peak milk production deviated from 4.10% in either direction.

No significant effect of Australian Breeding Value for daughter fertility (linear term only) on the risk of pregnancy loss adjusted for herd and cow age was observed within Holstein-Friesian cows (hazard ratio for a 1 unit increase in Australian Breeding Value for daughter fertility for cow: 1.07, 95% CI: 0.88-1.30, P=0.51; Table 2).

For models that required the inclusion of breed as a covariate based on the causal diagram, hazard ratio estimates were compared between when breed was fitted and not fitted (with more cows used when breed was not fitted). Hazard ratio estimates for peak daily litres with breed as a covariate were 1.024 and 1.003 for linear and quadratic terms, respectively, compared with 1.013 and 1.003 when breed was not fitted (Table 2), indicating that the estimates where breed was not fitted were not markedly confounded by breed. Hazard ratio estimates for milk fat concentration when breed was fitted as a covariate were 0.813 and 1.703 for linear and quadratic terms, respectively, which were similar to those when breed was not fitted (0.821 and 1.655, respectively; Table 2). Hazard ratio estimates for calving to conception categories when breed was fitted as a covariate were 0.625 and 1.037 for cows calved 61-120 days and cows more than 120 days calved at conception, respectively, compared with 0.763 and 0.961, respectively, when breed was not fitted; Table 3).

Discussion

We identified 7.8% of pregnancies lost after first confirmation of pregnancy within the interval day 35¬–161 after conception. Clinical mastitis and both low (<30) and high (>30) peak daily litres were risk factors for pregnancy loss.

The proportion of pregnancies that were lost in our study was more than twice the proportion lost across comparable risk periods in pasture-based cows in a New Zealand study. In that study, pregnancy loss frequency was 3.6% between (approximately) days 28 to 100 of pregnancy, with a final pregnancy loss frequency of 6.4% of pregnancies from day 28 to calving[5] which was still lower than our loss frequency over a shorter period. Other studies comparing reproductive performance of cows managed in similar systems in New Zealand and Australia have shown country-level differences in reproductive performance. These include a greater proportion of cows not returning to service within 24 days after AI amongst cows subsequently diagnosed as non-pregnant in Australian compared to New Zealand cows (49% versus 29%)[15]. A proportion of these cows are likely to have experienced early embryonic loss. However the pregnancy loss frequency between days 28 and 84 of gestation in pasture-fed cows in a 2002 Irish study of 7.2%[16] was similar to that observed in the current study. The individual herd pregnancy loss incidences ranged from 5.2% to 10.9% suggesting that herd differences are present and important. The current study did not aim to assess the repeatability of herd pregnancy loss incidence so only cows from a single mating period from each herd were included. A larger longitudinal study to better define the range of herd pregnancy loss incidences and to determine the repeatability of individual herd pregnancy loss incidences is warranted. Any herd experiencing a pregnancy loss incidence over all cows that become pregnant in excess of 10% would likely be under substantial financial stress.

The current study was not designed to assess the incidence of pregnancy loss in the first 35 days after conception but additional analyses indicate that this was also possibly substantial in the study herds. For each herd, of cows diagnosed as not becoming pregnant to their fixed-time AI, high proportions had also not returned to service within the first 24 days after those AIs (range between herds: 50% to 61%). It is likely that at a proportion of these cows in fact conceived to that insemination but subsequently experienced an early embryonic loss before the first pregnancy test was performed. Risk of pregnancy loss is higher in early compared to later stages of pregnancy[8].

Cows with clinical mastitis after conception had a 2.7-fold greater hazard of pregnancy loss than cows free from clinical mastitis. This was identical to the hazard ratio from a US study of pregnant cows followed for a period of 90 days after a clinical mastitis event that

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occurred within the first 45 days of gestation17. The New Zealand study (McDougall et al., 2005) also identified clinical mastitis as a risk factor for pregnancy loss (hazard ratio: 1.57). Systemic responses arising as a result of clinical mastitis may be inducing pregnancy loss in some cows with clinical mastitis. The clinical mastitis cumulative incidence in spring was within the expected range in herds with good mastitis control (each herd had spring bulk milk somatic cell counts below 250,000 cell/ml) and for herds with a large number of carryover cows18. However, some under-reporting may be present as, in one herd, no clinical mastitis events were reported. Such underreporting would tend to bias the estimated effect of clinical mastitis towards null, so it is likely that the true effect of clinical mastitis is larger than indicated by our estimate. However, some cases of clinical mastitis will have occurred after pregnancy loss occurred (and thus, could not have caused the pregnancy loss). This will have tended to bias the estimated effect of clinical mastitis towards overestimation.

We identified a strong curvilinear relationship between milk production and pregnancy loss. Cows producing less than or more than 30 litres per day at peak lactation were at greater risk of pregnancy loss compared with those producing about 30 litres. The asymmetry in the curvilinear nature of the relationship implies that cow pregnancy loss risk increases more for every additional litre of milk above a 30-litre lactation peak than for every additional litre of peak milk below a 30-litre lactation peak. Further work is required to determine whether the effect of peak daily litres that we observed is due to differences in nutrient balance in early lactation, is reflective of differences in genetic merit for milk production, is a combination of both, or has some other mechanism. An hypothesis has been proposed that, within pasture-fed cows, those of high genetic merit for milk production may be at increased risk of negative energy balance and consequently pregnancy loss than cows of more modest genetic merit19. However, if pregnancy loss has a genetic component cause, any effects of genetic merit for milk production on pregnancy loss will depend on the closeness of correlation between genetic merit for milk production and each of genetic merit for pregnancy loss in both the dam and the conceptus. The adverse effects of low peak daily litres may reflect effects of low body condition at calving and/or health disorders at calving and in early lactation, if these both reduce peak daily litres and increase risk of pregnancy loss.

Our results also provided some evidence for a curvilinear relationship between milk fat concentration at peak milk production and risk of pregnancy loss; risk was least in cows whose milk fat concentration at peak milk production was around 4.10%. The increased risk with high fat concentrations may be due to adverse effects of post-partum negative energy balance on pregnancy loss. Excessive negative energy balance increases risk of pregnancy loss[2]. Cows with severe negative energy balance after calving tend to mobilize more body fat and this can result in increased milk fat concentration relative to milk protein concentration20. The increased risk in cows with low peak lactation milk fat concentrations may reflect the presence of other health or metabolic conditions such as ruminal acidosis[21].

A review of pregnancy loss identified reduced circulating progesterone during the growth phase of the dominant follicle of pregnancy, increasing parity, increased loss of body condition from calving to breeding, uterine diseases and effects of other diseases such as mastitis as the predominant risk factors for pregnancy loss between days 28 and 608. It is not known whether most losses in this period are primarily due to death of the foetus or due to failure of the dam to maintain the pregnancy. Losses during the critical third period of pregnancy (28–60 days) as described by these authors as 'of substantial importance in determining reproductive efficiency of dairy herds' arising from the delayed return to cycling by affected cows and the substantive delay to any subsequent (successful) pregnancy. Thus, they conclude that there is a need to manage maternal health, cow nutrition, body condition and the maternal hormonal environment to minimise pregnancy losses during this period.

The low numbers of two-year-old cows in study herds is worthy of comment. The corporate entity managing each herd had a business model in which they sold both milk and rising two-year-old pregnant dairy heifers. This meant that few home-bred heifers entered the study herds with most replacements being purchased cows that had completed at least one lactation in another herd.

Conclusions

We observed pregnancies in Australian grazing dairy cattle for loss typically from 45 to 161 days after conception; 7.8% of pregnancies were lost. These cows are at very high risk of remaining non-pregnant at the end of the herd's mating period. Risk of pregnancy loss in grazing cows increases in cows that experience clinical mastitis after conception, and in cows whose peak milk yields are either below or above 30 litres per day (with risk increasing the further peak daily production is from 30 litres). The incidence of pregnancy loss in grazing dairy cows may be reduced if joint causes of low peak daily litres and pregnancy loss are removed (potentially including implementation of strategies to better manage cows producing high peak daily litres), and clinical mastitis incidence is reduced. The genetic effects in both the dam and the conceptus on pregnancy loss should also be investigated, to assess the extent to which currently used fertility breeding values are also capturing those effects. In summary, further research focusing on better defining and understanding the peak milk production effect, impact of breed and genotype and stresses such as mastitis on risk of pregnancy loss is warranted following our findings in this exploratory study.

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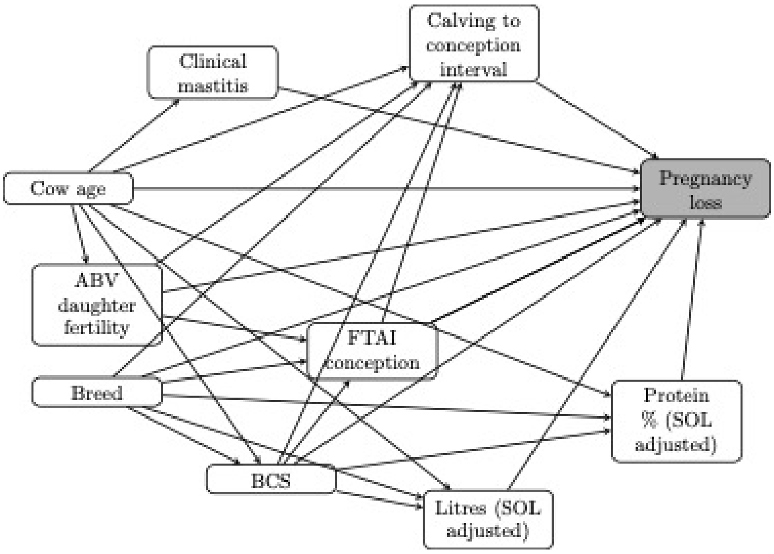

Figure 1: Causal diagram of pregnancy loss from the first confirmed pregnant pregnancy test to approximately day 161 of pregnancy; ABV: Australian Breeding Value; BCS: body condition score; FTAI conception indicates whether or not the conception was from the fixed time AI; Litres is estimated daily litres per cow at peak lactation, obtained by adjusting litres at the first milk recording after the start of the mating period for the cow's stage of lactation (SOL); Protein % is the estimated milk protein concentration at peak lactation.

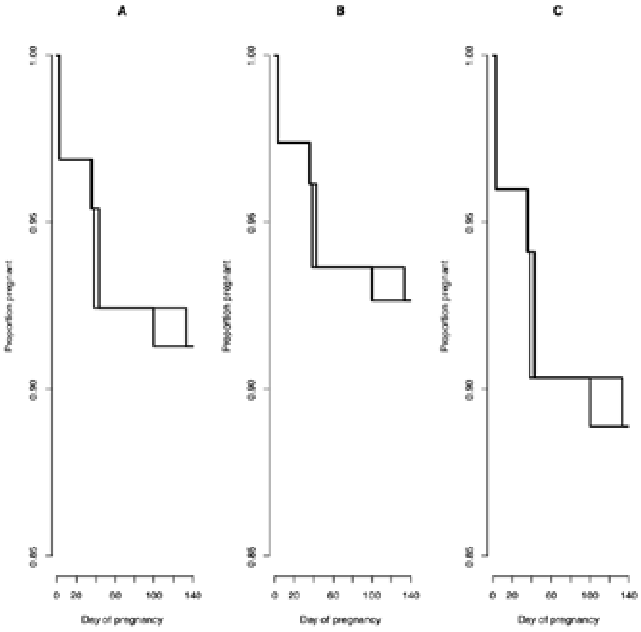

Figure 2: Interval-censored survival curves for cows peaking at 20 (plot A), 30 (plot B) and 40 (plot C) litres of milk per day. Interval censored time periods are indicated by boxed regions of the curves

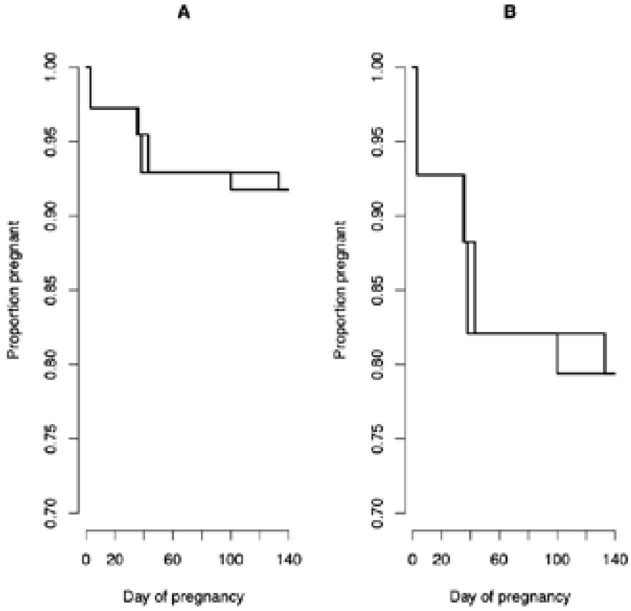

Figure 3: Interval-censored survival curves for cows free from clinical mastitis between the cow's first positive pregnancy diagnosis and its final pregnancy test or, for cows losing their pregnancy, to the first pregnancy test where the pregnancy loss was identified (plot A) and cows experiencing one or more cases of clinical mastitis in this period (plot B). Interval censored time periods are indicated by boxed regions of the curves boxed regions of the curves.

Continued ••→

Table 1: Numbers of cows included, numbers and proportions losing pregnancy by study exposure variable categories

Exposure variable	Level	Number (No. losing pregnancy)	Proportion losing pregnancy (95% CI)
Herd	Herd 1	302 (33)	0.109 (0.076-0.150)
	Herd 2	210 (11)	0.052 (0.026-0.092)
	Herd 3	237 (13)	0.054 (0.030-0.092)
	Herd 4	400 (33)	0.083 (0.057-0.114)
	Total	1149 (90)	0.078 (0.063-0.095)
Breed	Holstein-Friesian	357 (41)	0.115 (0.084-0.153)
	Jersey	8 (0)	0.000 (0.000-0.369)[1]
	Crossbreed	155 (14)	0.090 (0.050-0.147)
	Unknown	629 (35)	0.055 (0.039-0.077)
Australian Breeding Value for daughter fertility (Holstein-Friesian cows only)	<100	68 (3)	0.044 (0.009-0.124)
	100 to <104	142 (16)	0.113 (0.066-0.177)
	≥104	61 (7)	0.115 (0.047-0.222)
	Unknown	878 (64)	0.073 (0.057-0.092)
Age at calving (years)	2	59 (5)	0.085 (0.028-0.187)
	3	197 (5)	0.025 (0.008-0.058)
	4	254 (27)	0.106 (0.071-0.151)
	5+	638 (53)	0.083 (0.063-0.107)
	Unknown	1 (0)	-
Body condition score on day 1 of the herd's mating period (1 to 8 scale where 1 is thin and 8 is fat)	< 4.5	488 (40)	0.082 (0.059-0.110)
	4.5 to <5.0	508 (40)	0.079 (0.057-0.106)
	≥5	22 (1)	0.045 (0.001-0.228)
	Unknown	131 (9)	0.069 (0.031-0.126)
Calving to conception interval (days)	1-60	80 (8)	0.100 (0.044-0.188)
	61 to 120	575 (40)	0.070 (0.050-0.094)
	>120	493 (42)	0.085 (0.062-0.113)
	Unknown	1 (0)	-
Conception was to fixed time AI	Yes	375 (33)	0.088 (0.061-0.121)
	No	774 (57)	0.074 (0.056-0.094)
Peak daily milk litres per cow	<25	238 (16)	0.067 (0.039-0.107)
	25 to 35	685 (48)	0.070 (0.052-0.092)
	>35	224 (26)	0.116 (0.077-0.165)
	Unknown	2 (0)	-
Milk protein concentration at peak production (gm/100 mL milk)	≤3.00	229 (25)	0.109 (0.071-0.157)
	>3.00 to 3.50	726 (48)	0.066 (0.049-0.087)
	>3.50	191 (16)	0.084 (0.049-0.132)
	Unknown	3 (1)	0.333 (0.008-0.906)
Milk fat concentration at peak production (gm/100 mL milk)	≤3.50	158 (21)	0.133 (0.084-0.196)
	>3.50 to 4.50	719 (44)	0.061 (0.045-0.081)
	>4.50	269 (24)	0.089 (0.058-0.130)
	Unknown	3 (1)	0.333 (0.008-0.906)
Clinical mastitis[2]	No	1106 (36)	0.033 (0.060-0.092)
	Yes	43 (7)	0.163 (0.068-0.307)

1 Exact one-sided 97.5% confidence interval

2 One or more cases of clinical mastitis ('Yes') or none ('No') between the cow's first positive pregnancy diagnosis and its final pregnancy test or, for cows losing their pregnancy, to the first pregnancy test where the pregnancy loss was identified

Table 2. Hazard ratios and 95% confidence intervals for the total effects of continuous explanatory variables from separate interval-censored Cox proportional hazards models.

Potential risk factor	Pregnancy loss		No pregnancy loss		Hazard ratio[2]	95% CI	P
	No. cows[1]	Mean (SD)	No. cows[1]	Mean (SD)			
Australian Breeding Value for daughter fertility (Holstein-Friesian cows only)	41	103.0 (2.53)	316	102.5 (2.63)	1.07	0.88-1.30	0.52[3]
					[Adjusted for cow age and herd]		
Age at calving (years)	46	6.0 (2.9)	578	5.7 (2.5)	Linear: 1.04	0.95-1.13	0.73[4]
					Quadratic: 1.00	0.95-1.05	0.98[5]
					[Adjusted for herd]		
Cow body condition score on day 1 of herd's mating period	81	4.7 (0.22)	937	4.7 (0.20)	Linear: 1.05	0.30-3.64	0.77[4]
					Quadratic: 0.41	0.02-10.17	0.78[5]
					[Adjusted for cow age and herd][6]		
Peak daily milk litres per cow	90	30.8 (8.41)	1,057	29.9 (6.51)	Linear: 1.013	0.981-1.046	0.005[4]
					Quadratic: 1.003	1.000-1.006	0.007[5]
					[Adjusted for cow age, body condition score and herd]		
Milk protein concentration at peak production (gm/100 mL milk)	89	3.20 (0.30)	1,057	3.24 (0.30)	Linear: 0.94	0.36-2.47	0.98[4]
					Quadratic: 1.11	0.27-4.49	0.89[5]
					[Adjusted for cow age, body condition score and herd][7]		
Milk fat concentration at peak production (gm/100 mL milk)	89	4.07 (0.63)	1,057	4.11 (0.54)	Linear: 0.82	0.56-1.20	0.07[4]
					Quadratic: 1.66	1.13-2.42	0.03[5]
					[Adjusted for cow age, body condition score and herd][7]		

1 Number of cows used in model to estimate total effect of variable
2 Estimated total effect for a 1 unit increase in potential risk factor; adjusted for covariates as listed
3 Likelihood ratio test p-value
4 Likelihood ratio test p-value for linear and quadratic terms jointly
5 Likelihood ratio test p-value for quadratic term only (i.e. p-value for assessing the null hypothesis that the quadratic term coefficient is 0 when the linear term is already included in the model)
6 Breed and Australian Breeding Value for daughter fertility were also identified from the causal diagram as potential confounders but the effect of body condition score was estimated without adjustment for these as Australian Breeding Values were not comparable between breeds and data were not available for either variable for many cows
7 Breed was also identified from the causal diagram as a potential confounder but effects reported here were not adjusted for breed because breed data were not available for many cows

Table 3. Hazard ratio and 95% confidence interval for the total effects of categorical explanatory variables from separate interval-censored Cox proportional hazards models.

Potential risk factor	No. cows[1]	No. cows with pregnancy loss	% cows with pregnancy loss	Hazard ratio[2]	95% CI	P3
Breed						
Purebreed (i.e. Holstein-Friesian or Jersey)	365	41	9.0	Reference category		
Crossbreed	155	14	11.2	0.75	0.39-1.44	0.37
				[Adjusted for herd]		
Conception was to fixed time AI						
No	774	57	7.4	Reference category		
Yes	375	33	8.8	1.13	0.64-1.99	0.39
				[Adjusted for body condition score and herd][4]		
Calving to conception interval (days)						
1-60	80	8	10.0	Reference category		
61-120	575	40	7.0	0.76	0.24-2.44	0.58
>120	493	42	8.6	0.96	0.30-3.10	
				[Adjusted for cow age, body condition score and herd][4]		
Clinical mastitis[5]						
No	1,106	83	7.5	Reference category		
Yes	43	7	16.3	2.70	1.06-6.92	0.03
				[Adjusted for cow age and herd]		

1 Numbers of cows used in model to estimate total effect of variable
2 Estimated total effect; adjusted for covariates as listed
3 Likelihood ratio test p-value (joint likelihood ratio test p-value for calving to conception interval)
4 Breed and Australian Breeding Value for daughter fertility were also identified from the causal diagram as a potential confounder but the effects of conception to fixed time AI and calving to conception interval were estimated without adjustment for these as Australian Breeding Values were not comparable between breeds and data were not available for either variable for many cows
5 One or more cases of clinical mastitis ('yes') or none ('no') between the cow's first positive pregnancy diagnosis and its final pregnancy test or, for cows losing their pregnancy, to the first pregnancy test where the pregnancy loss was identified