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ORIGINAL RESEARCH

Risk factors for ultrasound-diagnosed endometritis and its impact on fertility in Scottish dairy cattle herds

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Abstract

Background: The aim of this study was to investigate the risk factors for and the impact of ultrasound-diagnosed endometritis (UDE) on lactating dairy cows' reproductive performance.

Methods: Data were analysed from 1123 Holstein and Holstein-Friesian cows from two Scottish dairy farms. A reproductive ultrasound examination was conducted on two occasions, at 43 ± 3 and 50 ± 3 days in milk (DIM), to screen for hyperechoic fluid in the uterus. Statistical analyses were performed using multivariable logistic regression modelling and Cox proportional hazards models.

Results: The overall incidence of UDE was 8.8% (99/1123). Risk factors for UDE included calving during autumn/winter seasons, increased parity and the presence of two or more diseases in the first 50 ± 3 days postpartum. The presence of UDE was associated with a reduced odds of pregnancy after all artificial inseminations up to 150 DIM.

Limitations: The retrospective design of this study led to some inherent limitations with the quality and quantity of data collected.

Conclusions: The findings of this study indicate which risk factors should be monitored in postpartum dairy cows to limit the impact of UDE on future reproductive performance.

INTRODUCTION

Postpartum uterine disease is a common problem in dairy cattle, with bacterial contamination of the uterine lumen occurring in 80%–90% of cows within 2 weeks of parturition.[1] The cow's immunity and capacity to limit disease severity in response to these pathogens will determine the outcome on uterine health.[2] Uterine infection creates a hostile uterine environment, disrupts endocrine signalling and alters ovarian function and oocyte development, all of which may lead to poor reproductive performance[3] and can compromise animal health and welfare.

Clinical endometritis (CE) is characterised by the presence of purulent or mucopurulent fluid within the uterine lumen at or beyond 21 days postpartum.[1] A number of methods have been described to diagnose CE; however, the reliability of and agreement between diagnostic tests is low.[4] One method commonly used in the diagnosis of CE is identification of purulent vaginal discharge (PVD). Vaginoscopy can be used to identify in situ PVD and visualise the cranial vagina, although its use under field conditions is limited.[5] Insertion of a gloved hand or vaginal device (Metricheck, Simcro, New Zealand) into the vagina provides a more detailed, ex situ method of assessing vaginal discharge. Interpretation of PVD must be performed with caution, as it is not possible to distinguish between animals with vaginitis or cervicitis and those with CE.[4] Dubuc et al.[6] found a poor correlation between animals with PVD and cytological endometrial inflammation, suggesting that PVD is not synonymous with CE and instead is a diagnosis in its own right. Transrectal ultrasonography is a rapid and readily available diagnostic tool used to identify pathological conditions of the uterus.[7] Diagnosis of CE can be made based on ultrasound assessment of uterine size, luminal shape and the presence and echogenicity of intraluminal fluid to guide diagnostic and prognostic decisions.[8,9] Recent work by Kelly et al.[10] reported 67% (33%–100%) sensitivity and 91% (86%–93%) specificity for ultrasound examination in the diagnosis of CE.

It is clear that the various diagnostic methods can assess different aspects of reproductive tract health, with individual pathologies being considered as unique diagnoses that may have differing impacts on reproductive performance.[4] Therefore, the term 'reproductive tract disease' (RTD) has been recently introduced, and will be used throughout this article, as a more accurate descriptor to encompass the broad range of uterine pathologies, including PVD and CE, that may occur after 21 days postpartum.[10]

Cows diagnosed with RTD have a 5%–25% reduction in their chance of pregnancy per artificial insemination (AI) compared with healthy cows.[10,11] In addition, RTD causes anovulation and prolonged luteal activity, which can lead to reduced expression of oestrus, in turn leading to lower submission rates to AI.[11,12] These factors combined may result in delayed pregnancy, with cows suffering from RTD shown to have a median time to pregnancy that is 32 days longer than healthy animals.[13] Moreover, cows with RTD have an increased probability of culling (73%–127%) due to poor reproductive performance when compared with healthy cows.[13,14]

The main risk factors for RTD can be linked to the host, the environment and the interaction between the two. Multiple cow-level risk factors have been recognised, including parity,[15,16] stillbirth,[17] twinning[15] and birth of a male calf.[17] Some of the postpartum diseases identified as contributing to the development of RTD are hypocalcaemia,[18] hyperketonaemia,[19] retained fetal membranes (RFM)[16,17] and metritis.[14,20] The compounding negative impact of developing multiple diseases in the postpartum period has been investigated for outcomes including milk production,[21] probability of pregnancy,[21] ovarian activity[22] and culling risk,[23,24] but not for the risk of developing RTD specifically. Extrinsic factors such as season,[24,25] farm environment[26] and calving factors (e.g., stillbirth or dystocia)[17] have also been shown to contribute to the development of RTD.

Early identification and management of these risk factors is required to limit the negative impact of RTD on subsequent reproductive performance. Despite a substantial body of literature on postpartum uterine disease, few studies outline the risk factors for and reproductive outcomes of RTD diagnosed using transrectal ultrasound.[8,10,15] The primary objective of this study was to identify the risk factors for ultrasound-diagnosed endometritis (UDE). The secondary objective was to investigate the impact of UDE on subsequent reproductive performance, specifically the impact of UDE on pregnancies at 60 days after the first postpartum AI and time to pregnancy after all AIs up to 150 days in milk (DIM). We hypothesised that the occurrence of any postpartum disease during the voluntary waiting period (up to 50 ± 3 DIM) would be associated with the development of UDE, with an increasing number of disease events being associated with an increased risk of UDE. Additionally, we hypothesised that the presence of UDE would be negatively associated with pregnancies at 60 days after the first postpartum AI and with pregnancy after all AIs up to 150 DIM.

MATERIALS AND METHODS

Study population

A dataset was available from a total of 1123 Holstein and Holstein-Friesian cows from two commercial dairy farms in central Scotland. Data were collected over a 16-month period, from October 2018 to May 2020, for a concurrent study comparing reproductive management strategies in lactating dairy cows. The methods outlined in this study received ethical approval from the University of Glasgow (Faculty Ethics and Welfare Committee, licence number 44a/18).

Both herds were housed all year round in cubicle sheds, separated into primiparous and multiparous groups, the latter of which were further separated into milking groups dependent on yield. Cows were milked three times daily at 8-hour intervals. Cows were fed ad libitum grass silage-based total mixed ration, formulated to meet or exceed the requirements for maintenance and milk production.[27] Animals had ad libitum access to water. During the study period, Farms 1 and 2 were milking 765 and 580 cows, respectively. The mean 305-day mature-equivalent milk production (305ME) calculated from Cattle Information Service monthly milk recordings was 13,279 kg (standard deviation [SD] 1967 kg) for Farm 1 and 10,770 kg (SD 1194 kg) for Farm 2.

Postpartum health monitoring

Incidence of twins, stillbirths (death within 24 hours of birth) and sex of the calf were recorded by farm staff prior to or at parturition. Both herds received weekly routine reproductive management visits from veterinarians from the University of Glasgow. All cows between 1 and 7 DIM were presented for postpartum disease screening, including a full clinical examination and manual vaginal examination.

Clinical hypocalcaemia was recorded by farm staff when a cow displayed clinical signs of muscle weakness and tremors, cold extremities and eventually recumbency. RFM were recorded by farm staff in cows where fetal membranes were not expelled by 12 hours after parturition.[28] Ketonuria was measured by veterinarians using commercial urine dipsticks containing nitroprusside (KetoStix, Bayer Diagnostics Europe, Dublin, Ireland), where a colour change of 'trace' or greater was recorded as a positive case. Animals with watery, foetid, red–brown uterine discharge, pyrexia (rectal temperature 39.2°C or higher) and clinical signs indicative of systemic illness, including poor rumen fill and reduced milk yield, were diagnosed by veterinarians with puerperal metritis, whereas those with foetid uterine discharge in the absence of pyrexia and ill health were diagnosed with clinical metritis.[1] For the purpose of recording, cases of clinical and puerperal metritis were grouped together into one diagnosis of metritis. Left displacement of the abomasum (LDA) was diagnosed if a resonant 'ping' was audible on percussion and auscultation of the left flank between the 9th and 12th intercostal spaces. Lameness, mastitis and bovine respiratory disease (BRD) were monitored and recorded weekly by veterinarians until the time of reproductive tract examination at 50 ± 3 DIM. Animals were body condition scored by veterinarians during the postpartum examination (1–7 DIM) and at the first reproductive tract ultrasound examination (43 ± 3 DIM) using a scale of 1–5 in increments of 0.25, as described by Edmonson et al.[29] All diseases were treated by farm staff or veterinarians using standard treatments outlined in the farm's herd health plan.

Production data, specifically the average daily milk weight produced during the fourth week of lactation (W4MK), were recorded for individual cows using the farm's management software (DairyComp 305; Valley Agricultural Software, Tulare, CA, USA). Increased W4MK is an indicator of production success early in lactation and has been positively associated with improved early reproductive performance.[30]

Reproductive management

Both farms followed a reproductive management programme that had a voluntary waiting period of 50 days and included a combination of oestrus detection and synchronisation protocols. All cows were inseminated between 50 ± 3 and 70 ± 3 DIM based on natural oestrus expression, detected via visual observation by farm staff for 30 minutes three times daily. Any animal not detected in oestrus after 70 ± 3 DIM was enrolled in a hormone synchronisation protocol with fixed-time AI at 80 ± 3 DIM. Sexed Holstein semen was used for the first three inseminations in primiparous cows, after which beef or conventional semen was used. Sexed semen was not used in multiparous cows, which instead were inseminated with either conventional or beef semen.

Uterine health monitoring and pregnancy diagnosis

A 7.5-MHz linear transducer (Easy-Scan II, IMV Imaging, UK) was used to conduct a reproductive tract examination on two occasions, at 43 ± 3 and 50 ± 3 DIM. Both ovaries were assessed, and the presence and size of follicles and corpora lutea were recorded. The uterus was scanned in cross-section to assess for the presence of intraluminal fluid, following the method outlined by Mee et al.[8] All animals found with 0.2 cm or more of mixed echogenicity fluid in the uterine lumen on at least one of the ultrasound examinations, regardless of severity, were recorded as having UDE. Vaginal examination to assess for the presence of PVD was not performed simultaneously with the ultrasound examination.

Cows were presented at 29–35 days after AI for pregnancy diagnosis using the transrectal ultrasound described above. Fetal age, fetal viability and the presence of twins were assessed as described by Fricke.[31] Additionally, all cows diagnosed as pregnant at their initial pregnancy diagnosis were re-examined at 60–66 days after AI for pregnancy diagnosis confirmation. Cows recorded as positive in the first pregnancy diagnosis and negative in the second pregnancy diagnosis were recorded as having a pregnancy loss.

Statistical analysis

All data were organised and summarised in Microsoft Excel (Version 2011, Microsoft Corporation) and exported for analysis to Stata 16 (StataCorp. 2019; Stata Statistical Software: Release 17; StataCorp, College Station, TX, USA).

Stillbirth, ketonuria, RFM, metritis, mastitis, lameness, hypocalcaemia, LDA and BRD were dichotomised (yes = 1; no = 0) for the analysis. Births were categorised into female calf (0), male calf (1) and twins (2). Parity was categorised into primiparous and multiparous cows. Body condition score (BCS) at calving and at 43 ± 3 DIM were categorised as BCS 2.5 or less or BCS greater than 2.5. BCS loss between calving and 43 ± 3 DIM was categorised as 'yes' in cows losing 0.5 or more points and 'no' in cows that lost less than 0.5 points and maintained or gained BCS. Seasons were grouped into spring/summer and autumn/winter. Any diagnosis of hypocalcaemia, RFM, ketonuria, metritis, lameness, mastitis, BRD and LDA occurring before 50 ± 3 DIM was considered a disease event. Due to a low incidence of individual disease events, cows were grouped into no disease (0), one disease (1) and two or more diseases (2) categories for the multivariable logistic regression modelling. ANOVA was used to compare W4MK for the UDE groups. Chi-squared statistics were used to compare distributions of cows on categorical risk factors for UDE groups. Season of calving, parity and farm were offered as potential confounders to all statistical models. All biologically plausible interactions were tested in the analysis, including parity × disease, farm × disease, season of calving × disease, farm × parity and season of calving × farm. Pearson correlation coefficients were calculated to measure the strength of linear association between pairs of variables that were included in the statistical model.

Prior to multivariable logistic regression modelling to assess reproductive performance in this retrospective study, a power sample calculation was performed.[32] We obtained sufficient power (type II error = 0.83; type I error = 0.05) to detect a 15% reduction in pregnancy detection at 60 days following the first postpartum AI (41%–26%) between the healthy (n = 1024) and UDE (n = 94) groups, with an incidence of UDE of 9%. A second power sample calculation was performed prior to Cox proportional hazard analysis of reproductive performance.[32] Using a total of 1118 cows (healthy = 1024; UDE = 94) available for the analysis, we obtained a power of 0.89 (type II error) with a type I error of 0.05 to detect a hazard ratio (HR) for time to pregnancy after all AIs up to 150 DIM of 0.7, with an incidence of UDE of 9%.

In the reproductive analysis, diagnosis of UDE and pregnancy at 60 days following the first postpartum AI were considered as main outcomes in the multivariable logistic regression models. Farm and UDE were included as fixed effects in all models for pregnancy at 60 days following the first postpartum AI, regardless of effect size or statistical significance. Additionally, Kaplan–Meier survival curves were used to visualise the time to pregnancy after all AIs up to 150 DIM. Healthy and UDE groups were compared with a pairwise log-rank test. The hazard of time to pregnancy after all AIs up to 150 DIM was analysed using Cox proportional hazard models with the same risk factors used in the logistic regression model. Statistical significance in all models was considered as a p-value of 0.05 or less.

For all analyses, all variables were tested using univariable logistic regression and included in the multivariable analysis if the p-value was less than 0.3. Multivariable logistic regression models were constructed via backwards variable elimination using the likelihood ratio test and Akaike's information criterion (AIC), choosing the model with the smallest AIC. Model fit was assessed using the Hosmer–Lemeshow goodness of fit test. Statistical significance in all models was considered as a p-value of 0.05 or less. Model fit was considered acceptable in all statistical models presented. Additionally, log–log plots were used to assess time dependency assumptions prior to constructing the Cox proportional hazards models.

RESULTS

Descriptive statistics

Data were analysed from a total of 1123 cows (700 cows from Farm 1 and 423 cows from Farm 2). Descriptive statistics are presented in Table 1. The overall incidence of UDE across the dataset was 8.8% (99/1123). The incidence of UDE was higher on Farm 1 (11.7%; 82/700) than on Farm 2 (4%; 17/423) ($p < 0.01$) and was higher in multiparous cows (11.1%; 78/701) than in primiparous cows (5.0%; 21/422) ($p < 0.01$). There was no significant interaction between parity and disease ($p = 0.46$), farm and disease ($p = 0.78$), season of calving and disease ($p = 0.51$), farm and parity ($p = 0.10$) and season of calving and farm ($p = 0.72$). The proportion of animals diagnosed with UDE was greater ($p <$ 0.01) at 43 ± 3 DIM (77.8%) than at 50 ± 3 DIM (22.2%).

TABLE 1 Descriptive statistics from chi-squared analysis determining the relationship between risk factors and the development of ultrasound-diagnosed endometritis (UDE) for 1123 Scottish dairy cows

	Healthy (%)	UDE (%)	*p*-Value
Farm			<0.01
1 (n = 700)	88.3	11.7	
2 (n = 423)	96.0	4.0	
Lactation			<0.01
Primiparous (n = 422)	95.0	5.0	
Multiparous (n = 701)	88.9	11.1	
Season of calving			0.02
Spring/summer (n = 618)	93.0	7.0	
Autumn/winter (n = 505)	88.9	11.1	
Stillbirth			0.48
Yes (n = 52)	88.5	11.5	
No (n = 1071)	91.3	8.7	
Birth			<0.01
Single female (n = 589)	92.5	7.5	
Single male (n = 498)	90.6	9.4	
Twins (n = 36)	77.8	22.2	
Ketonuria			<0.01
Yes (n = 83)	78.3	21.7	
No (n = 1040)	92.2	7.8	
Retained fetal membranes			<0.01
Yes (n = 71)	73.2	26.8	
No (n = 1052)	92.4	7.6	
Metritis			<0.01
Yes (n = 85)	76.5	23.5	
No (n = 1038)	92.4	7.6	
Mastitis			0.71
Yes (n = 29)	93.1	6.9	
No (n = 1094)	91.1	8.9	
Lameness			0.61
Yes (n = 70)	92.9	7.1	
No (n = 1053)	91.1	8.9	
Hypocalcaemia			0.59
Yes (n = 3)	100	0	
No (n = 1120)	91.2	8.8	
Left displaced abomasum			0.34
Yes (n = 12)	83.3	16.7	
No (n = 1111)	91.3	8.7	
Bovine respiratory disease			0.53
Yes (n = 4)	100.0	0	
No (n = 1119)	91.1	8.9	
No. of diseases			<0.01
0 (n = 841)	93.2	6.8	
1 (n = 208)	88.9	11.1	
≥2 (n = 74)	74.3	25.7	
BCS at 43 ± 3 DIM			0.48
BCS ≤ 2.5 (n = 126)	92.9	7.1	
BCS > 2.5 (n = 997)	91.0	9.0	
W4MK (SD)	39.8 (±11.4)	44.7 (±11.9)	<0.01

Abbreviations: BCS, body condition score; DIM, days in milk; SD, standard deviation; W4MK, average daily milk weight produced during the fourth week of lactation.

In addition, 12 of 99 (12.1%) cows were diagnosed with UDE at both ultrasound examinations.

UDE was more frequently found in cows calving in the autumn/winter seasons ($p = 0.02$). Twenty-two percent of animals that calved twins developed UDE, compared with 9.4% of animals calving a single male calf and 7.5% of animals calving a single female calf ($p < 0.01$). There was a numerically higher occurrence of UDE ($p = 0.24$) in cows calving a male calf than in cows calving a female calf (9.4% vs. 7.5%). The incidence of twins was significantly influenced by farm, with 83.3% (30/36) of twin calvings from Farm 1 ($p < 0.01$), and by parity, with 94.4% (34/36) of twin calvings in multiparous cows ($p < 0.01$).

The incidence of UDE increased with increasing W4MK ($p < 0.01$), although W4MK was higher on Farm 1 (42.7 SD ± 11.5) than on Farm 2 (35.6 SD ± 9.9) ($p < 0.01$) and also higher in multiparous cows (45.7 SD ± 10.1) when compared to primiparous cows (31.2 SD ± 7.2) ($p < 0.01$).

There was an association between the incidence of UDE and ketonuria ($p < 0.01$), RFM ($p < 0.01$) and metritis ($p < 0.01$). Farm and parity had an impact on the development of these diseases, where ketonuria ($p < 0.01$), RFM ($p < 0.01$) and metritis ($p = 0.02$) were associated with increased parity, and farm was linked to RFM with 77.5% (55/71) of cases on Farm 1 ($p < 0.01$). Season had no impact on the development of ketonuria ($p = 0.13$), RFM ($p = 0.47$) or metritis ($p = 0.05$). The presence of multiple diseases was significantly associated with the development of UDE, where 25.7% (19/74) of animals with two or more diseases were diagnosed with UDE compared to 11.1% (23/208) and 6.8% (57/841) of animals with one disease or no disease, respectively ($p < 0.01$).

Multivariable logistic regression for the risk factors for UDE

Correlations between all significant variables associated with the development of UDE were considered low, aside from W4MK, which had a moderate degree of correlation ($r = 0.609$, $p < 0.05$) with parity and was therefore excluded from subsequent logistic regression models.

The final logistic regression model (Table 2) found farm (adjusted odds ratio [AOR] = 0.27; confidence interval [CI] = 0.16–0.47; $p < 0.01$), multiparity (AOR = 2.23; CI = 1.33–3.75; $p < 0.01$) and calving during the autumn/winter season (AOR = 1.73; CI = 1.12–2.67; $p < 0.01$) to be associated with an increased risk of developing UDE. When all diseases occurring before 50 ± 3 DIM were grouped to assess the impact of multiple postpartum disease events, it was found that cows with two or more diseases were 2.62 times more likely to be diagnosed with UDE (95% CI = 1.29–5.28; $p < 0.01$) than cows with one disease, and 4.02 times more likely to be diagnosed with UDE than cows with no disease recorded (95% CI = 2.16–7.47; $p < 0.01$). However, there was no difference in the incidence of UDE when comparing cows with one disease and no disease recorded ($p = 0.11$).

Multivariable logistic regression for the impact of UDE on reproductive performance

Within the group of cows diagnosed with UDE, 5.1% (5/99) were excluded from breeding due to unresolved UDE and were subsequently culled and thus excluded from the reproductive analysis. The results of the final multivariable logistic regression model for pregnancy at 60 days following the first postpartum AI are shown in Table 3. Multiparous cows were less likely to be pregnant at 60 days after the first postpartum AI than primiparous cows (AOR = 0.53; 95% CI = 0.41–0.68; $p < 0.01$). A greater BCS (greater than 2.5) was associated with a greater likelihood of pregnancy at 60 days following the first postpartum AI (AOR = 1.62; 95% CI = 1.06–2.46; $p < 0.03$). Pregnancy at 60 days after the first postpartum AI was more likely on farm 2 than on farm 1 (AOR = 1.43; 95% CI = 1.10–1.85; $p < 0.01$). There was a negative association between UDE and pregnancy at 60 days following the first postpartum AI, which tended towards significance (AOR = 0.64; 95% CI = 0.39–1.03; $p = 0.07$).

Survival analysis for the impact of UDE on reproductive performance

The results from Cox proportional hazard modelling are presented in Table 4. Farm did not have a significant impact on the hazard of pregnancy after all AIs up to 150 DIM (HR = 1.08; 95% CI = 0.93–1.25; $p = 0.29$). Multiparous cows had a decreased hazard of pregnancy after all AIs up to 150 DIM compared to primiparous cows (HR = 0.62; 95% CI = 0.54–0.72; $p < 0.01$). A greater BCS (greater than 2.5) was associated with an increased hazard of pregnancy after all AIs up to 150 DIM (HR = 1.36; 95% CI = 1.03–1.80; $p = 0.03$). The hazard of pregnancy after all AIs up to 150 DIM was lower for cows with UDE than for healthy cows (HR = 0.73; 95% CI = 0.56–0.97; $p = 0.03$) (Figure 1).

DISCUSSION

The incidence of UDE in this study was 8.8%. This is lower than studies where alternative methods, such as vaginal examination, were used to diagnose RTD but agrees with other work employing ultrasound in the diagnosis of RTD. Plöntzke et al.[33] reported a prevalence of 35% in cows examined for the presence of PVD between 18 and 38 DIM. Similarly, LeBlanc et al.[13] reported a prevalence of 16.9% when diagnosis of RTD was made using vaginoscopy to assess cervical diameter and the presence of PVD at 20–33 DIM. The higher prevalence of RTD found in these studies may reflect the fact that PVD encompasses

TABLE 2 Results of multivariable logistic regression model for risk factors related to the development of ultrasound-diagnosed endometritis for 1123 Scottish dairy cows

	Coefficient	Adjusted odds ratio	95% confidence interval	Standard error	*p*-Value
Intercept	−4.77				<0.01
Farm					
1 ($n = 700$)	Referent				
2 ($n = 423$)	−4.63	0.27	0.16−0.47	0.08	<0.01
Parity					
Primiparous ($n = 422$)	Referent				
Multiparous ($n = 701$)	3.03	2.23	1.33−3.75	0.59	<0.01
Season					
Spring/summer ($n = 618$)	Referent				
Autumn/winter ($n = 505$)	2.49	1.73	1.12−2.67	0.38	0.01
No. of diseases					
0 ($n = 841$)	Referent				
1 ($n = 208$)	1.59	1.54	0.90−2.61	0.41	0.11
≥2 ($n = 74$)	4.39	4.02	2.16−7.47	0.03	<0.01

TABLE 3 Results of multivariable logistic regression model for factors influencing the odds of pregnancy at 60 days after the first postpartum artificial insemination for 1118 Scottish dairy cows

	Coefficient	Adjusted odds ratio	95% confidence interval	Standard error	*p*-Value
Intercept	−0.75				0.45
Farm					
1 ($n = 695$)	Referent				
2 ($n = 423$)	2.70	1.43	1.10−1.85	0.19	<0.01
Parity					
Primiparous ($n = 420$)	Referent				
Multiparous ($n = 698$)	−5.05	0.53	0.41−0.68	0.07	<0.01
BCS at 43 ± 3 DIM					
BCS ≤ 2.5 ($n = 125$)	Referent				
BCS > 2.5 ($n = 993$)	2.24	1.62	1.06−2.46	0.35	0.03
Uterine status					
Healthy ($n = 1024$)	Referent				
UDE ($n = 94$)	−1.88	0.64	0.39−1.03	0.16	0.07

Abbreviations: BCS, body condition score; DIM, days in milk; UDE, ultrasound-diagnosed endometritis.

animals with vaginitis and cervicitis as well as those with CE.[4] Additionally, these studies were conducted at an earlier stage of lactation and could therefore include animals that have the potential to self-cure, given that the odds of self-cure are known to be higher in cows where PVD is diagnosed at 21–27 DIM.[34] In contrast, studies employing transrectal ultrasound at a similar number of DIM (range 25–49 DIM) to the current study reported a similar prevalence of RTD. Specifically, using an ultrasound scoring system with a cut-off point of more than 3 mm of intrauterine fluid in cows at 28–41 DIM, Barlund et al.[35] reported a prevalence of RTD of 10%. Kelly et al.[15] reported an 8.7% prevalence of RTD in cows with more than 2 mm of intrauterine fluid identified during a single pre-breeding reproductive tract ultrasound examination at 25–49 DIM. The lower incidence of RTD reported when using transrectal ultrasound at a later stage of lactation is thought to be due to the exclusion of animals that have self-cured or those with PVD from aetiologies other than endometritis.[10]

Metritis and RFM were associated with increased odds of UDE. This supports the findings of previous studies that showed that uterine disease in the immediate postpartum period was a major risk factor for developing RTD.[14,16,17,20] Although cows could suffer from RFM, metritis and endometritis as individual diseases, there is a proven link between the development of these pathologies whereby the risk of metritis may be higher in cows previously diagnosed with RFM,

TABLE 4 Results of Cox proportional hazard model for the hazard of pregnancy after all artificial inseminations up to 150 days in milk (DIM) for 1118 Scottish dairy cows

	Coefficient	Hazard ratio	95% confidence interval	Standard error	*p*-Value
Farm					
1 ($n = 695$)	Referent				
2 ($n = 423$)	1.04	1.08	0.93–1.25	0.08	0.29
Parity					
Primiparous ($n = 420$)	Referent				
Multiparous ($n = 698$)	−6.58	0.62	0.54–0.72	0.04	<0.01
BCS at 43 ± 3 DIM					
BCS ≤ 2.5 ($n = 125$)	Referent				
BCS > 2.5 ($n = 993$)	2.17	1.36	1.03–1.80	0.19	0.03
Uterine status					
Healthy ($n = 1024$)	Referent				
UDE ($n = 94$)	−2.19	0.73	0.56–0.97	0.10	0.03

Abbreviations: BCS, body condition score; UDE, ultrasound-diagnosed endometritis.

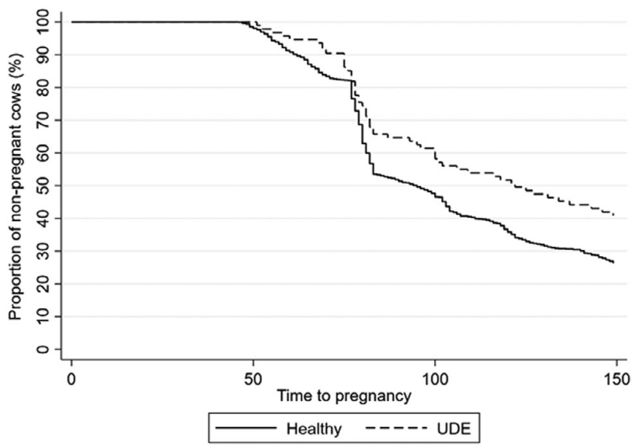

FIGURE 1 Kaplan–Meier survival curves for time to pregnancy after all artificial inseminations (AIs) up to 150 days in milk (DIM) for 1118 Scottish dairy cows. Animals followed a 50-day voluntary waiting period with AI based on natural oestrus expression between 50 ± 3 and 70 ± 3 DIM prior to hormonal synchronisation for fixed-time AI at 80 ± 3 DIM. UDE, ultrasound-diagnosed endometritis

and in turn, the risk of endometritis is increased when cows are diagnosed with metritis.[36] The correlation between metritis and RFM in this dataset was low and both were independently associated with UDE.

Postpartum negative energy balance has been linked to impaired uterine immune function and has been reported by multiple authors as a risk factor for RTD.[19,20,37] Similar to these studies, we identified ketonuria as a risk factor for the development of UDE. Low BCS in the prepartum period has been directly linked to the development of RTD.[38] Excessive BCS loss from the dry period to early lactation has been associated with poor health in early lactation, including an increased incidence of metritis, RFM and hyperketonaemia.[39–41] Limitations in data collection meant that loss of BCS between dry off and lactation was not analysed in the present study. Changes between body condition at calving and 43 ± 3 DIM were not associated with UDE, indicating that these measures of body condition alone are not useful when considering the risk factors for UDE. It is suggested, based on the findings of this and previous studies, that changes in body condition should be measured from the point of dry off to fully understand the effect of body condition loss and negative energy status on the development of UDE.

The presence of other disease events (LDA, mastitis, BRD and lameness) within the first 50 ± 3 DIM was not associated with UDE. Although these diseases occurring in the periparturient period have been shown to have a negative impact on cow health and reproductive performance,[42,43] this is likely not reflected in this study due to their low incidence in the dataset.

We found that cows diagnosed with two or more diseases before 50 ± 3 DIM were more likely to be diagnosed with UDE than cows with no disease recorded. The presence of multiple adverse health events early in lactation has been linked by other authors to poor reproductive and productive performance, including prolonged postpartum anovulation, reduced pregnancy rates and reduced milk production.[21–24] Postpartum dairy cattle commonly suffer from poor immunocompetence and systemic inflammation, and the presence of infectious and/or metabolic diseases in the postpartum period has been shown to further challenge the cow's immune system by causing impaired leukocyte function and dysfunctional or unregulated inflammatory responses.[44,45] Diseased animals suffering from these metabolic and immunological challenges are at increased risk of succumbing to subsequent disease and developing comorbidities.[46,47] In particular, more prolonged or severe postpartum disease will cause prolonged uterine inflammation and limit the cow's ability to control the inevitable bacterial contamination of the uterus following parturition, therefore increasing the risk of developing uterine disease.[2,44] The increased odds of UDE observed in animals with an increasing number of negative health events prior to 50 ± 3 DIM is thought to be a direct reflection of the magnitude of metabolic stress and reduced immune capacity of more severely diseased animals. In a practical setting, it is suggested that animals suffering from multiple disease events during the voluntary waiting period would benefit from an ultrasound examination at 43 ± 3 and 50 ± 3 DIM in order to diagnose and treat UDE prior to breeding.

We found that multiparous cows had greater odds of developing UDE. Parity and RTD have a recognised 'U-shaped' association.[15,48,49] Studies have shown that primiparous animals may be at increased risk of RTD due to dystocia and associated genital tract damage, leading to increased susceptibility to uterine infection.[17,24,34] On the other hand, older cows with three or more lactations have been shown to be more likely to develop RTD for reasons including poor vulval conformation,[17] increased postpartum uterine infection[16] and increased risk of clinical or subclinical hypocalcaemia leading to delayed uterine involution.[50,51] It is believed that the higher odds of UDE in multiparous cows in this study may be explained by these factors, alongside calving factors such as the increased rate of twinning in older animals.[52] The low incidence of UDE in primiparous cows in this study may be explained in part by farm management, where primiparous animals were housed separately from multiparous animals and thus may have suffered from less hierarchical stress with consequently improved health.[53] Additionally, farm breeding policies of inseminating primiparous cows with sexed semen resulted in a low incidence of dystocia with a lower probability of calving trauma and associated infection postpartum.[17]

Cows calving twins have a greater risk of dystocia due to an abnormal presentation and a higher incidence of RFM compared to cows having single calves.[54] In various previous studies, twin birth was identified as a risk factor for the occurrence of postpartum uterine disease due to its relationship with dystocia, consequent retention of fetal membranes and development of metritis.[17,34] In agreement with these studies, we also found the birth of twins to be a significant risk factor in the development of UDE.

Calving in the autumn/winter was associated with an increased odds of UDE. This is in agreement with recent work by Pinedo et al.,[24] who demonstrated an increased incidence of RTD during the cold seasons in cows from large, housed dairy herds in the United States. However, these results conflict with various other studies, where an increased incidence of RTD was found during warm seasons in both grazing and housed cattle.[25,34] Studies linking seasonality to poor uterine health are often conducted in temperate regions where the temperature–humidity index is high, and they typically indicate heat stress during the transition period to be a key risk factor in the development of uterine pathologies.[25,55,56] Heat stress in Scottish and UK dairy cows has a relatively low rate of occurrence.[57] We hypothesised that the impact of season on UDE observed in this study may instead be linked to husbandry differences during the autumn/winter, when Scottish farms typically have a higher stocking density and greater environmental contamination.

Compared with healthy cows, cows with UDE had a numerically lower rate of pregnancy at 60 days following the first postpartum AI and a significantly lower hazard of pregnancy after all AIs up to 150 DIM. Various studies have reported a negative association between RTD, time to pregnancy and pregnancy rates following first postpartum AI.[11,58] The reduction in the chances of pregnancy per AI observed in animals with UDE is thought, in part, to be related to disturbed postpartum ovarian activity, as RTD has been shown to cause prolonged luteal phases and poor follicular function.[12,59] Additionally, poor pregnancy per AI rates observed in cows with UDE may be related to a poor uterine environment during early pregnancy, as it is known that embryo development and attachment are negatively impacted by the presence of endometrial inflammation and fluid accumulation in animals with RTD.[3] The reduced hazard of pregnancy after all AIs up to 150 DIM observed in animals with UDE in this study demonstrates the wide impact of RTD on fertility, as pregnancy status at 150 DIM is known to be a composite trait that reflects the efficiency of submission for AI and conception rate regardless of farm management.[60] It was also found that 5.1% of cows in the UDE group were classified as 'do not breed' animals and were subsequently culled later in their lactation, which is similar to findings of previous studies that have shown that animals with RTD are at higher risk of culling, particularly for poor reproductive performance.[13,14,61]

CONCLUSION

Increased parity, cooler weather in the autumn/winter and an increasing number of disease events occurring during the voluntary waiting period (up to 50 ± 3 DIM) were associated with increased odds of UDE. Cows with UDE have a reduced hazard of pregnancy after all AIs up to 150 DIM. Cows suffering from one or more negative health events early in lactation are at increased risk of developing UDE and should be monitored to allow early intervention and treatment in hopes of reducing subsequent negative reproductive performance outcomes.

AUTHOR CONTRIBUTIONS

All authors contributed to the conceptualisation of the study. Ciara McKay analysed the data and drafted the original manuscript in collaboration with and supervised by Richard Vazquez. Lorenzo Viora, John Cook and Katharine Denholm had substantial input into revising the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The methods outlined in this study received ethical approval from the University of Glasgow (Faculty Ethics and Welfare Committee, licence number 44a/18).

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