Late embryonic losses in supplemented grazing lactating dairy cows: Risk factors and reproductive performance

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ABSTRACT

The main objective of this study was to evaluate the risk factors for late embryonic loss (LEL) in supplemented grazing dairy cows. Additional objectives were to assess the incidence of LEL and its association with the reproductive performance of cows. A data set containing productive, reproductive, and health records of 13,551 lactations was used. A retrospective case-control study involving 631 cows with LEL (cases) and 2,524 controls (4 controls per case within each study year) was run. A case of LEL was defined when the embryo had no heartbeat or there was evidence of detached membranes or floating structures including embryo remnants by ultrasonography (US) at 28 to 42 d post-artificial insemination (AI), whereas a non-case was defined as a cow diagnosed with positive pregnancy by US 28 to 42 d post-AI and reconfirmed as pregnant 90 $\pm$ 7 d post-AI. Four controls per case were randomly selected from the non-cases with a temporal matching criterion (± 3 d around the date of the fecundating AI of the case). Multivariable logistic models were offered with the following predictors: year of LEL (2011 through 2015), season of LEL (summer vs. fall vs. winter vs. spring), parity (1 vs. 2 vs. ≥ 3), uterine disease (UD), non-uterine disease (NUD), body condition score at parturition, body condition score at 28 to 42 d post-AI (BCS-LEL), days in milk (DIM), and daily milk yield (MY). Statistical significance was set at $P < 0.05$ and a tendency was set at $P \leq 0.10$. We found that 4.7, 22, and 23% of cows had LEL, UD, and NUD, respectively. Cases tended to have higher daily MY than controls (32.5 vs. 31.8 kg); also, cases had much

longer calving to pregnancy interval (226 vs. 118 d), lower hazard of pregnancy [hazard ratio = 0.39, 95% confidence interval (CI) = 0.35–0.43], and higher odds for non-pregnancy [odds ratio (OR) = 2.89, 95% CI = 2.37–3.54] than controls. We found that the odds for LEL increased with parity number (OR = 2.48, 95% CI = 1.99–3.08 for parity ≥ 3) and with BCS-LEL < 2.50 (OR = 1.81, 95% CI = 1.33–2.47). Conversely, the odds for LEL decreased with BCS-LEL > 3.00 (OR = 0.70, 95% CI = 0.53–0.91). The odds for LEL increased with UD (OR = 1.23, 95% CI = 1.01–1.49), NUD (OR = 1.24, 95% CI = 1.01–1.54), DIM (OR = 1.03, 95% CI = 1.00–1.05), and daily MY (OR = 1.14, 95% CI = 1.04–1.25) in univariable models only. Finally, the odds for LEL were not associated with year, season, DIM, and body condition score at parturition. In conclusion, LEL is associated with extended calving to pregnancy interval, and among its risk factors are parity number and BCS-LEL.

Key words: late embryonic loss, reproductive performance, clinical disease, milk yield, grazing dairy cow

INTRODUCTION

The establishment and maintenance of pregnancy in lactating dairy cows is the paramount objective of dairy farms in order to remain profitable over time (De Vries, 2006). Several factors can affect reproduction in dairy cows, such as extended postpartum anovulatory period, poor estrus detection, low pregnancy per insemination, and high pregnancy loss (Santos et al., 2009). Pregnancy can be lost at any time during entire gestation, and it is classified as embryonic loss at < 42 d post-AI or as fetal loss at ≥ 43 d post-AI (Evans and Walsh, 2011). Most of the losses occur during the first 60 d of gestation at embryonic or early fetal stages (Wiltbank et al., 2016). Early embryonic loss (0–24 d

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post-AI) detection is not practical under commercial conditions because it requires the measurement of pregnancy-associated proteins (Engelke et al., 2015). Conversely, late embryonic loss (LEL; 25–42 d post-AI) becomes easier to diagnose due to the routine use of ultrasound examination for early pregnancy diagnosis (around 30 d post-AI). The literature about pregnancy loss, including LEL and early fetal loss (i.e., from about 25 up to 84 d post-AI) reported that the proportion of cows that lose their pregnancies varies from 7.2 to 29% (Silke et al., 2002; Diskin et al., 2006; Gábor et al., 2008; Santos et al., 2009; Abdalla et al., 2017).

Among the proposed risk factors for pregnancy loss (i.e., embryo loss and early fetal loss) are year and season (García-Ispuerto et al., 2006; Khan et al., 2013), parity number (Silke et al., 2002; Sartori et al., 2004; Lee and Kim, 2007; Bamber et al., 2009; El-Tarabany, 2016), BCS (Silke et al., 2002; Ribeiro et al., 2013; Trevisi, 2018), milk production (Silke et al., 2002; Diskin et al., 2006; Leroy et al., 2008), and clinical diseases (Perry et al., 2005; Celi et al., 2011; Mokhtari et al., 2016a; Ribeiro et al., 2016). Therefore, there is a need for studies specially designed to address the issue of LEL. The main objectives of this study were to assess the incidence of and risk factors for LEL in supplemented grazing dairy cows. An additional goal was to assess its association with reproductive performance. The hypotheses to test were that the incidence of LEL in supplemented grazing dairy cows is similar to that previously reported and that parity number, clinical diseases, low BCS, high milk yield (MY), and summer increase the odds for LEL.

MATERIALS AND METHODS

Herd Management

The study was carried out in a commercial dairy herd located in Carlos Casares, Province of Buenos Aires, Argentina (35°37' S, 61°22' W), with a land base of 2,000 ha and approximately 2,600 Holstein dairy cows. Parturient transition cows were kept on a dry lot and monitored for signs of calving by farm employees. Calving occurred year-round. Fresh cows were kept on a dry lot for 3 d. Then, lactating cows were at pasture and supplemented with concentrates and silage. The total diet of lactating dairy cows consisted of 50% pasture, 25% concentrate, and 25% corn silage. Pastures consisted mainly of alfalfa and winter annual grasses (ryegrass). Cows grazed different paddocks in the morning and afternoon. Corn-based concentrate (60% cornmeal and 40% soybean pellets) was equally fed 4 times per day, 2 times in the parlor during milking and 2 times mixed with corn silage. Corn silage was delivered twice

per day in the paddocks. The average MY was 9,600 kg per lactation.

The voluntary waiting period was set at 50 DIM. All cows were detected on heat twice daily (during morning and afternoon milkings) and were artificially inseminated. Estrus detection was assisted by using tail painting. Cows were considered in estrus when more than 50% of tail paint was removed. An Ovsynch protocol was used for cows not bred within 70 DIM. Pregnancy diagnosis was performed by transrectal ultrasonography (US) at 28 to 42 d post-AI using a 7.5-MHz linear transducer (Esaote Tringa Linear Vet, Pie Medical, Maastricht, the Netherlands). Reconfirmation of pregnancy was performed by US at 90 ± 7 d post-AI. Culling was mainly based on cow MY.

Disease Definition

All diseases were diagnosed by one of the authors (GD) with the exception of retained fetal membrane, clinical mastitis, and lameness, which were diagnosed by trained farm personnel. Retained fetal membrane was defined as when fetal membranes were not expelled by 24 h after calving (Kelton et al., 1998). Metritis was defined as cows having fetid vaginal discharge <21 DIM (Giuliodori et al., 2013a). Clinical endometritis was defined as the presence of pus in vaginal discharge >21 DIM (Sheldon et al., 2006). Pyometra was defined as the accumulation of purulent material within the uterine lumen in the presence of a persistent corpus luteum and a closed cervix (Sheldon et al., 2006). Clinical mastitis was defined as cows having abnormal milk secretion (e.g., clots, flakes, or watery secretion) from 1 or more quarters (Kelton et al., 1998). Clinical lameness was defined as cows having an episode of abnormal gait attributable to either the foot or the leg regardless of etiology or duration (Kelton et al., 1998). Finally, LEL was defined as when the embryo had no heart-beat or there was evidence of detached membranes or floating structures including embryo remnants by US (López-Gatius and García-Ispuerto, 2010).

Animal Selection and Study Design

A data set with data for all the lactations started between January 1, 2011, and December 31, 2015 (13,551 cows), was extracted from commercial software (Dirsa S.A., Gonnet, Argentina) and used in this study. This study was approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Sciences, National University of La Plata, Argentina (code no. 66-2-17T).

Only lactations with valid reproductive and milk production data were eligible to be included in the study.

Valid records were those having (1) a pregnancy diagnosis 28 to 42 d post-AI, (2) at least 1 BCS <30 DIM (BCS on a scale of 1 to 5; Ferguson et al., 1994), (3) at least 1 BCS ± 15 d relative to the date of pregnancy-LEL diagnosis, and (4) at least 1 monthly milk check 30 to 45 d post-AI. Additionally, for lactations without a record of LEL, a pregnancy reconfirmation at 90 ± 7 d post-AI was required.

A retrospective case-control study was carried out to assess risk factors for LEL (Rothman et al., 2008). A case was defined as a cow diagnosed with LEL 28 to 42 d post-AI by transrectal US. A non-case was defined as a cow with positive pregnancy diagnosis 28 to 42 d post-AI by transrectal US that remained pregnant when rechecked at 90 ± 7 d post-AI. This was done to exclude cows that had a pregnancy loss after being diagnosed pregnant at 28 to 42 d post-AI. In those cows with more than 1 event of LEL ($n = 42$) within the same lactation, only the first event was included in the study. A temporal matching (± 3 d around the date of the fecundating AI of the case) was used to randomly select 4 controls per case out of the available non-cases within each study year. Therefore, cases not accompanied by 4 controls were not included in the study.

Descriptive Data

The data set consisted of 13,551 records and had a total of 642 cases of LEL. Four cases of LEL were excluded because of failure with temporal matching (not accompanied by 4 controls) and 7 were excluded because they were diagnosed outside the time window (28–42 d post-AI). Therefore, 631 cases were included in the analysis together with 2,524 controls (out of 7,588 non-cases).

Statistical Analysis

The association of LEL (cases vs. controls) with MY was estimated with PROC GLIMMIX of SAS (SAS/STAT version 9.4, SAS Institute Inc., Cary, NC) with normal distribution and identity link function adjusted by the quadratic effect of DIM (the mean when MY was measured was 148 DIM). The association of LEL with reproductive performance was assessed through calving to pregnancy interval, hazard of pregnancy, and odds for non-pregnancy. The calving to conception interval was estimated using PROC LIFETEST of SAS. The hazard of pregnancy was estimated with PROC PHREG of SAS. Finally, the odds for non-pregnancy were estimated with PROC GLIMMIX of SAS with binomial distribution and logit link function.

The risk for LEL was evaluated by logistic models with PROC GLIMMIX of SAS with binomial distribution

and logit link function. Potential risk factors were first assessed as fixed effects in univariable models and then offered to multivariable models if $P < 0.15$. Assessed risk factors were year of LEL (2011 through 2015), season of LEL (summer vs. fall vs. winter vs. spring), parity (1 vs. 2 vs. ≥ 3), uterine disease (UD; yes vs. no; included retention of fetal membranes, metritis, clinical endometritis, and pyometra), non-uterine diseases (NUD; yes vs. no; included clinical mastitis and clinical lameness that occurred between AI and pregnancy diagnosis by US 28–42 d post-AI), BCS at parturition (BCS-PAR; <2.75 vs. 2.75–3.25 vs. >3.25), BCS at 28 to 42 d post-AI (BCS-LEL; <2.50 vs. 2.50–3.00 vs. >3.00), and all first-order interactions. Also, DIM at LEL and MY were included as continuous predictors. Modeling in multivariable logistic models was performed using the manual backward elimination method with an exclusion criterion set at $P > 0.1$. Statistical significance was set at $P < 0.05$ and a tendency was set at $P \leq 0.10$.

RESULTS

Descriptive Analysis

In the data set ($n = 13,551$), incidence of LEL, UD, and NUD was 4.7, 22.0, and 23.0%, respectively. Cases conceived at similar DIM [median (top and bottom quartiles)] as controls [118 (75–204) and 118 (81–182), respectively], and the different cases experienced LEL on that gestation, whereas the controls did not.

Association of LEL with MY and Reproductive Performance

Cases tended to have higher MY than controls (32.5 ± 0.36 vs. 31.8 ± 0.17 kg; $P = 0.10$). Also, cases had longer calving to pregnancy interval [median (95% CI); 226 (208–238) vs. 118 (115–122) d; $P < 0.01$; Figure 1] and lower hazard of pregnancy than controls (hazard ratio = 0.385; 95% CI = 0.346–0.429; $P < 0.01$). Finally, cases had higher odds for non-pregnancy than controls (odds ratio = 2.894; 95% CI = 2.368–3.536; $P < 0.01$) given that the percentage of open cows was 26 (164/631) and 11% (281/2,524) in cases and controls, respectively.

Risk Factors for LEL

The odds for LEL were not associated with year ($P = 0.99$), season ($P = 0.98$), or BCS-PAR ($P = 0.95$; Table 1). The odds for LEL were higher in cows in parity ≥ 3 than in cows in parity 1 ($P < 0.01$; Table 2). The odds for LEL increased in cows with BCS-LEL <2.50

Figure 1. Calving to pregnancy interval in supplemented grazing dairy cows ($n = 3,155$) from a commercial farm in Argentina experiencing late embryonic loss (LEL; cases, $n = 631$) or not (controls, $n = 2,524$). Cows with late embryonic loss had longer calving to pregnancy interval [median (95% CI)] than herd mates [226 d (208–238) vs. 118 d (115–122); $P < 0.01$]. Cows with LEL had lower hazard of pregnancy than herd mates without LEL (hazard ratio = 0.385; 95% CI = 0.346–0.429; $P < 0.01$). LEL was defined as when the embryo had no heartbeat or there was evidence of detached membranes or floating structures including embryo remnants by ultrasonography at 28 to 42 d post-AI. Cows with no LEL (controls) were defined as cows with a positive pregnancy diagnosis performed by ultrasonography at 28 to 42 d post-AI and reconfirmed as pregnant at 90 ± 7 d post-AI. Four controls per case were randomly selected with temporal matching (± 3 d around the date of the fecundating AI of the case).

and decreased in cows with BCS-LEL >3.0 ($P < 0.01$; Table 2). The odds for LEL were not associated with UD, NUD, DIM, and MY in the multivariable model (Table 2) even though these variables were associated with the risk in univariable models (Table 1). Finally, all first-order interactions had $P > 0.1$.

DISCUSSION

Incidence of LEL

Our results showed that 4.7% of cows had LEL between 28 and 42 d post-AI. Our finding is similar to that reported by Silke et al. (2002), who found a rate of 3.2% by using US in the same period (28–42 d post-AI). Santos et al. (2009) detected a decrease of 5.2 percentage points in conception rate from 38.3% on d 30 post-service to 33.1% 28 d later because of LEL and early fetal loss. Other authors have detected pregnancy losses of 22% by US between 30 and 70 d post-AI (Abdalla et al., 2017) and of 16.3% by using blood progesterone concentration between 30 and 60 d post-AI (Gábor et al., 2008). Differences in study design such as the timing of the diagnosis and assessed time window (28–42,

Table 1. Univariable logistic models assessing risk factors for late embryonic loss¹ (LEL) in supplemented grazing dairy cows ($n = 3,155$) from a commercial farm in Argentina

Item	OR ²	95% CI	P-value
Year of LEL			
2011	Referent		0.99
2012	0.965	0.689–1.352	
2013	0.984	0.715–1.354	
2014	0.992	0.717–1.371	
2015	0.958	0.688–1.335	
Season of LEL			
Summer	Referent		0.98
Fall	1.036	0.815–1.317	
Winter	1.039	0.817–1.323	
Spring	1.054	0.807–1.377	
Parity			
1	Referent		<0.01
2	1.505	1.188–1.907	
≥ 3	2.423	1.956–3.001	
BCS-PAR ³			
2.75–3.25	Referent		0.95
<2.75	1.032	0.837–1.273	
>3.25	1.037	0.754–1.427	
BCS-LEL ⁴			
2.50–3.00	Referent		<0.01
<2.50	2.016	1.488–2.731	
>3.00	0.821	0.631–1.069	
UD ⁵			
No	Referent		0.04
Yes	1.225	1.010–1.487	
NUD ⁶			
No	Referent		0.04
Yes	1.246	1.011–1.535	
DIM ⁷	1.025	1.004–1.046	0.02
Daily MY ⁸	1.136	1.036–1.246	0.01

¹Defined as when the embryo had no heartbeat or there was evidence of detached membranes or floating structures including embryo remnants by ultrasonography at 28 to 42 d post-AI.

²Odds ratio for LEL.

³BCS (5-point scale) during the first 30 DIM.

⁴BCS (5-point scale) 30 d before the diagnosis of pregnancy or LEL.

⁵Uterine disease detected postpartum (i.e., retained fetal membranes, metritis, clinical endometritis, and pyometra).

⁶Non-uterine disease (i.e., clinical mastitis and clinical lameness) diagnosed between AI and diagnosis of pregnancy or LEL.

⁷DIM at the diagnosis of pregnancy or LEL (performed 28–42 d post-AI). Odds are expressed as the increment of 21 d over the mean (148 d).

⁸Daily milk yield in the last monthly milk check before the diagnosis of pregnancy or LEL (performed 28–42 d post-AI). Odds are expressed as the increment of 1 SD (8.49 kg) over the mean (32.04 kg/d). This association was controlled by DIM and DIM $\times$ DIM.

30–58, or even 30 up to 84 d post-AI) and diagnostic tool used (US vs. progesterone measurement) make comparison among different studies difficult.

Association of LEL with Reproductive Performance

Cows that had an LEL event conceived 108 d later than the control cows, which was further delayed if we compare that increment with the delay associated with metritis (20 d; Giuliadori et al., 2013a), clinical

endometritis (30–70 d; Plöntzke et al., 2011; Giuliadori et al., 2013b, 2017), or subclinical endometritis (30 d; Madoz et al., 2013). With LEL, it is necessary to get the cows pregnant twice in the same lactation.

Risk Factors for LEL

We found that the odds for LEL were associated with parity and BCS-LEL, supporting the proposed hypothesis that the odds for LEL increase with parity number and low BCS. Our finding that parity was associated with the odds for LEL is in agreement with previous reports (Lee and Kim, 2007; El-Tarabany, 2016). However, other studies have failed to find this association (Silke et al., 2002; Bamber et al., 2009). A possible explanation could be that multiparous cows have lower plasma progesterone and estradiol concentrations than primiparous cows (Sartori et al., 2004; Borchardt et al., 2017), which could be associated with higher odds for LEL.

Our results showed that BCS-LEL (but not BCS-PAR) was also associated with the odds for LEL. Our result is in agreement with Ribeiro et al. (2016), who found that low BCS at AI was negatively associated with pregnancy on d 32 post-AI. In addition, Silke et

al. (2002) found that cows that lost BCS had a higher proportion of LEL than herd mates that kept or gained BCS postpartum. Conversely, Mokhtari et al. (2016b) and Silke et al. (2002) failed to find this association. A possible explanation for the link between low BCS and the risk for LEL could be through lipolysis, which leads to high nonesterified fatty acid (NEFA) concentration. During the past several decades, many direct toxic effects of NEFA have been demonstrated on many cells such as granulosa cells (Vanholder et al., 2005), oocytes (Leroy et al., 2011), and developing embryos (Leroy et al., 2010). In addition, high NEFA concentration could lead to insulin resistance, decreasing the sensitivity of the ovary to LH and FSH (Lucy, 2007).

We found that the odds for LEL were not associated with UD and NUD. This finding is in agreement with Mokhtari et al. (2016b), who did not find any association between UD and embryo and fetal loss 30 to 68 d post-AI. Conversely, Ribeiro et al. (2016) reported higher embryonic and fetal loss (8.9% more) in cows with clinical diseases, and others found that clinical mastitis and clinical lameness are associated with increased risk for fetal death (Risco et al., 1999; Santos et al., 2004; Alawneh et al., 2011). We also found that MY was not associated with the odds for LEL, which agrees with Silke et al. (2002) and Diskin et al. (2006), who did not find any association between MY or milk energy output by 120 DIM and embryonic loss. Conversely, Wiltbank et al. (2001) reported that high-producing cows had a higher risk for embryonic or fetal death. Finally, we found no association between year and season with the odds for LEL. Conversely, other studies have reported that embryonic loss 21 to 30 d post-AI is associated with high temperature–humidity index (García-Ispuerto et al., 2006) and that implantation failure in early pregnancy is associated with heat stress during summer (Khan et al., 2013).

One of the main strengths of the study is that this is the first case-control study assessing risk factors for LEL, whereas previous studies are cohort studies. Rothman et al. (2008) stated that case-control studies are a more efficient version of corresponding cohort studies to assess risk factors when a low proportion of the population is affected (Rothman et al., 2008). Another strength is that it involves a high number of cases of LEL that were directly detected in situ by US at the time of pregnancy check (i.e., the embryo was dying or recently dead), whereas previous studies were run on smaller data sets and most of them diagnosed the loss at pregnancy reconfirmation. A final strength is that it reports data about LEL in highly supplemented grazing dairy cows, whereas previous studies mostly involved confined cows, and only 1 included fully grazed cows. The main limitations are that (1) it is very likely that

Table 2. Final multivariable logistic model assessing risk factors for late embryonic loss¹ (LEL) in supplemented grazing dairy cows (n = 3,155) from a commercial farm in Argentina²

Item	OR ³	95% CI	P-value
Parity			
1	1		<0.01
2	1.531	1.207–1.942	
≥3	2.475	1.991–3.076	
BCS-LEL ⁴			
2.50–3.00	1		<0.01
<2.50	1.811	1.329–2.467	
>3.00	0.695	0.531–0.910	

¹Defined as when the embryo had no heartbeat or there was evidence of detached membranes or floating structures including embryo remnants by ultrasonography at 28 to 42 d post-AI.

²Predictors offered to the multivariable model were parity, BCS-LEL, uterine disease, non-uterine disease, DIM, daily milk yield, and first-order interactions. Uterine disease (i.e., retained fetal membranes, metritis, clinical endometritis, and pyometra) was removed from the multivariable model (odds ratio, OR = 1.090; 95% CI = 0.886–1.341; $P = 0.42$; not shown) and from Table 2. Non-uterine disease (i.e., clinical mastitis and clinical lameness) diagnosed between AI and diagnosis of pregnancy or LEL was removed from the multivariable model (OR = 1.129; 95% CI = 0.913–1.398; $P = 0.26$) and from Table 2. DIM (28–42 d post-AI; mean of 148 DIM) was removed from the multivariable model (OR = 1.009; 95% CI = 0.988–1.030; $P = 0.32$) and from Table 2. Daily milk yield in the last monthly milk check before the diagnosis of pregnancy or LEL, performed 28 to 42 d post-AI (mean of 148 DIM), was removed from the multivariable model (OR = 0.960; 95% CI = 0.863–1.069; $P = 0.46$) and from Table 2. All first-order interactions had $P > 0.1$.

³Odds ratio for LEL.

⁴BCS (5-point scale) 30 d before the diagnosis of pregnancy or LEL.

the incidence of LEL is underestimated because cows were assessed for LEL only once between 28 and 42 d post-AI with a time window of 3 d where the dead embryo could be seen by US, (2) it is very likely that the association between disease and odds for LEL is underestimated because subclinical diseases (i.e., subclinical endometritis, subclinical mastitis, subclinical lameness) were not included, and (3) this case-control study was carried out in only 1 commercial dairy farm.

CONCLUSIONS

The odds for LEL are higher in multiparous cows and in cows with low BCS compared with their counterparts. We also conclude that LEL is negatively associated with reproductive performance given that cows that experience LEL must get pregnant a second time in the same lactation, thus extending the calving to pregnancy by 108 d.

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