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Risk Factors for Late Embryonic Mortality in Dairy Cows

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Abstract : We determined the risk factors for late embryonic mortality in dairy cows. We diagnosed pregnancy at 31 days and then confirmed the diagnosis at 45 days after artificial insemination (AI) *via* ultrasonography. The presence of an embryo with a heartbeat was the criterion for a positive pregnancy diagnosis. A diagnosis of late embryonic mortality was made when there was no positive sign of pregnancy in cows previously diagnosed as pregnant. The overall incidence of late embryonic mortality among 3,695 pregnancies was 6.9%. Logistic regression analysis revealed that herd size, AI month, synchronization protocol, and postpartum disease were important risk factors for late embryonic mortality. Herd size > 100 (odds ratio [OR]: 0.66, $p < 0.05$) and 50-100 lactating cows (OR: 0.63, $p < 0.01$) had lower risks of late embryonic mortality than herd size < 50 lactating cows. Cows inseminated during May-July had a higher risk (OR: 1.49, $p < 0.05$) of late embryonic mortality than cows inseminated during February-April. Cows inseminated after estrus following $PGF_{2\alpha}$ treatment also had a higher risk (OR: 1.77, $p < 0.001$) of late embryonic mortality than cows inseminated following natural estrus. Lastly, cows with postpartum disease tended to have a higher risk (OR: 1.26, $p < 0.1$) of late embryonic mortality than cows without postpartum disease. In conclusion, late embryonic mortality associated with the herd size, AI month, synchronization protocol, and postpartum disease in dairy cows.

Key words **:** dairy cows, late embryonic mortality, risk factors, ultrasonography.

Introduction

Embryo mortality is a major cause of economic loss for dairy producer (7). Late embryonic mortality in dairy cattle is defined as pregnancy loss between gestational days 24 and 42, which defines the end of the differentiation period, whereas pregnancy loss prior to gestational day 24 indicates early embryonic mortality (5). A previous study investigated embryonic loss in Holstein cows in 44 herds in France and determined the rates of early and late embryonic mortality after the first artificial insemination (AI) to be 31.6% and 14.7%, respectively (12). Although the rate of late embryo mortality is lower than that of early embryo loss, late embryonic mortality delays the time to pregnancy, resulting in enormous economic losses (2,20). The advent of ultrasonography for the diagnosis of early pregnancy has allowed researchers to determine the time and rate of late embryonic mortality in cattle, which is approximately 12.8% (range: 3.2% to 42.7%) in dairy cows (23).

During implantation, uterine blood flow increases as the conceptus undergoes rapid development and any deficiencies in placental development lead to inadequate embryo development, with the result that unhealthy embryos must be eventually eliminated (27). As observed in the more commonly *in vitro* produced embryos or embryos derived from nuclear transfer, abnormalities in placental development also underlie the high rate of pregnancy loss during the late embryonic stage (27). Together with these pathophysiological findings on the causes of abnormal conceptus development, the identification of the risk factors for late embryonic mortality might help to establish strategies that improve embryo survival and reproductive efficiency.

Various factors, such as heat stress, a change in the body condition score, the insemination protocol, diseases such as retained placenta and pyometra, the sire, maternal age or parity, the uterine environment relating to progesterone, and the farm, can associate with pregnancy loss during different embryonic or/and fetal periods (3,6,14-16,19,24,26). However, the risk factors for late embryonic mortality involving a large population of pregnant dairy cows have not been defined. Moreover, the risk factors for late embryonic mortality might vary among different geographies, regions or countries because of differences in general management, environment (climate and weather), and herd control. Therefore, the objective of this study was to determine the risk factors for late embryonic mortality in Korean dairy herds.

Materials and Methods

Herds

This study was performed on 14 Holstein dairy farms, located in Chungcheong Province. Farms had an average of 53 lactating cows (range: 25 to 130). The cows were maintained in a loose housing system, fed a total mixed ration, and milked twice daily. The mean milk yield ranged from 9,000 to 11,500 kg per cow per year. During this study period, the average monthly air temperature and relative humidity ranged from 3.5 to17.8°C and 62.5% to 76.2% in

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the spring (March to May), 20.3 to 26.6ºC and 72.7% to 89.5% in the summer (June to August), 6.0 to 21.3ºC and 68.6% to 89.4% in the autumn (September to November), and −2.7 to −3.2ºC and 64.5% to 82.4% in the winter (December to February), respectively.

Health and reproductive management

Cows received regular reproductive health checks every 2 weeks, which included an examination of the ovarian structures and uterus by transrectal palpation and ultrasonography (Tringa Linear VET Ultrasound scanner fitted with a 5.0 MHz array transducer, Esaote Pie Medical, Maastricht, the Netherlands). Postpartum diseases were diagnosed and treated until recovery, pregnancy or culling. Retained placenta was defined as the retention of the fetal membrane for > 24 h. Cows diagnosed with retained placenta were not treated. Abomasal displacement was diagnosed by a pinging sound upon abdominal auscultation and all cases were corrected by surgery. Endometritis was diagnosed 4 weeks postpartum by examining for the presence of a cloudy discharge and an enlarged uterus *via* rectal examination and ultrasonography. The cows with endometritis were treated with an injection of cloprostenol (500 μg), a $PGF_{2\alpha}$ analogue (Estrumate, MSD Animal Health, Seoul, Korea) or one intrauterine infusion of 2% povidone-iodine solution (Betadine solution, Korea Pharma Co., Ltd., Hwasung, Korea), and retreated if necessary. Ovarian cysts were diagnosed by ultrasonography at 4, 6, and 8 weeks postpartum. A diagnosis of follicular cyst was based on the presence of ovarian structures with an internal diameter greater than 25 mm and a wall thickness less than 3 mm. Luteal cysts were diagnosed if the wall was greater than 3 mm in thickness in the absence of a normal corpus luteum. Cows diagnosed with ovarian follicular cysts were treated with an injection of gonadorelin (100 μg), a GnRH analogue (Godorel, Uni-Biotech Co., Ltd., Yesan, Korea). Cows diagnosed with luteal cysts were treated with an injection of cloprostenol (500 μg).

The voluntary waiting period from calving to the first AI was 50 days. Cows with estrus beyond the voluntary waiting period were inseminated according to the a.m.-p.m. rule. In addition to estrous detection, a herd reproductive management program was employed. Estrus synchronization was achieved with an injection of $PGF_{2\alpha}$ im or the Ovsynch program. The Ovsynch program involved the administration of GnRH on day 0, $PGF_{2\alpha}$ on day 7, and GnRH on day 9. Cows displaying estrus after $PGF_{2\alpha}$ im received AI according to the a.m.-p.m. rule, whereas those who underwent the Ovsynch program received a timed AI.

Pregnancy diagnosis and detection of late embryonic mortality

Pregnancy was diagnosed at 31.2 ± 0.1 days (range: 27-35 days) and then confirmed at 45.2 ± 0.1 days (range: 43-48 days) after AI *via* ultrasonography. The presence of an embryo with a heartbeat by ultrasonography was the criterion for the pregnancy diagnosis (13). The diagnosis of late embryonic mortality was made when there was no positive sign of pregnancy in cows previously diagnosed as pregnant (18,21).

Data collection and statistical analysis

This study included 3,695 pregnancies from 14 dairy herds

Table 1. Descriptive statistics after the analysis of the risk factors for late embryonic mortality using data from 3,695 pregnancies in 14 dairy herds

Variable	Level	No. of cows		
		Pregnant	Late embryonic mortality	%
Herd size	< 50 lactating cows	1,305	115	8.8
	50-100 lactating cows	1,706	99	5.8
	> 100 lactating cows	684	41	6.0
Cow parity	Primiparous	1,260	78	6.2
	Multiparous	2,435	177	7.3
AI month	February-April	938	59	6.3
	May-July	857	77	9.0
	August-October	863	58	6.7
	November-January	1,037	61	5.9
Synchronization protocol	Natural estrus	1,875	108	5.8
	$PGF_{2\alpha}$	1,175	119	10.1
	Ovsynch	645	28	4.3
Postpartum disease[a]	No	1,714	106	6.2
	Yes	1,981	149	7.5

[a]Postpartum disease includes retained placenta, abomasal displacement, endometritis, and ovarian cyst.

Table 2. Odds ratio (OR) and variables included in the final logistic regression model for the risk factors for late embryonic mortality using data from 3,695 pregnancies in 14 dairy herds

Variable	Adjusted OR	95% CI	*p*-value
Herd size			< 0.01
< 50 lactating cows	Reference		
50-100 lactating cows	0.63	0.479-0.840	< 0.01
> 100 lactating cows	0.66	0.451-0.955	< 0.05
AI month			< 0.05
February-April	Reference		
May-July	1.49	1.049-2.139	< 0.05
August-October	1.07	0.734-1.564	> 0.1
November-January	0.95	0.657-1.382	> 0.1
Synchronization protocol			<0.0001
Natural estrus	Reference		
$PGF_{2\alpha}$	1.77	1.341-2.323	< 0.001
Ovsynch	0.69	0.445-1.054	< 0.1
Postpartum disease[a]			
No	Reference		
Yes	1.26	0.968-1.637	< 0.1

[a]Postpartum disease includes retained placenta, abomasal displacement, endometritis, and ovarian cyst.

from 2009 to 2015. The parameters that were recorded for each cow were as follows: herd, cow parity, dates of AI and pregnancy diagnosis, late embryonic mortality, insemination protocol (AI following natural estrus or $PGF_{2\alpha}$ im, or the Ovsynch program), and any postpartum diseases (including retained placenta, abomasal displacement, endometritis, and ovarian cyst).

Data were expressed as the means ± standard error of the means. For statistical analyses, cow parity was defined as either primiparous or multiparous. Herd size was grouped as < 50, 50-100, and > 100 lactating cows. Table 1 shows the descriptive statistics after the analysis of the risk factors for late embryonic mortality in dairy cows. Statistical analyses were performed using the SAS program (version 9.4, SAS Inst., Cary, NC, USA).

To determine the risk factors for late embryonic mortality, logistic regression involving the LOGISTIC procedure was used. The logistic regression model included the herd size, cow parity, any postpartum disease (including retained placenta, abomasal displacement, endometritis, and ovarian cyst), AI month, and synchronization protocol, and the interactions between these variables. Backward stepwise regression was used in all models, and elimination was performed based on the Wald statistic criterion when $p > 0.20$. The odds ratio (OR) and 95% confidence interval (CI) were computed by logistic regression. Results were presented as proportions and OR with their respective 95% CIs. A p-value ≤ 0.05 was considered significant and $0.05 < p < 0.1$ was considered as a tendency toward a significant difference.

Results

The overall incidence of late embryonic mortality among 3,695 pregnancies in 14 dairy farms was 6.9%. The final model revealed that the herd size ($p < 0.01$), AI month ($p < 0.05$), and synchronization protocol ($p < 0.0001$) significantly affected the incidence of late embryonic mortality in dairy herds, while the effect of postpartum disease was less significant ($p < 0.1$) (Table 2). However, cow parity had no effect ($p > 0.1$). Herd size > 100 (OR: 0.66, $p < 0.05$) and 50-100 lactating cows (OR: 0.63, $p < 0.01$) had lower risks of late embryonic mortality than herd size < 50 lactating cows. Cows inseminated during May-July had a higher risk (OR: 1.49, $p < 0.05$) for late embryonic mortality than cows inseminated during February-April. Cows inseminated after estrus following $PGF_{2\alpha}$ treatment also had a higher risk (OR: 1.77, $p < 0.001$), whereas cows inseminated following the Ovsynch program tended to have a lower risk (OR: 0.69, $p < 0.1$) of late embryonic mortality than cows inseminated following natural estrus. Cows with postpartum disease (retained placenta, abomasal displacement, endometritis, or ovarian cyst) tended to have a higher risk (OR: 1.26, $p < 0.1$) of late embryonic mortality than cows without postpartum disease.

Discussion

Increased late embryonic mortality associated with the herd size (< 50 lactating cows), the AI month (May-July), the synchronization protocol ($PGF_{2\alpha}$ treatment prior to AI), and postpartum disease in dairy cows. The results of this study suggest that improvement of herd management by including facilities that reduce heat stress and incorporating effective reproductive and health controls might help to prevent late embryonic mortality in dairy cows.

The overall incidence of late embryonic mortality was 6.9% among 3,695 pregnancies in this study, which agrees with that (6.7% to 7.7%) reported in previous studies (8,17,22). However, it was lower than the incidence rate (9.1% to 17%) reported by others (1,4,11). Larger herd size > 50 lactating cows (5.8-6.0%) had a lower risk of late embryonic mortality than herd size < 50 lactating cows (8.8%) in the present study. The reason about differences in the incidence of late embryonic mortality according to herd size is not clear in this study. However, we assume that dairy farmers with a larger herd size could reduce the late embryonic mortality by provision of better facilities (including cooling equipment system during hot season), bunk space, and farm management under field conditions. Our results agree with a previous study (11) reporting that pregnancy losses between gestational days 28-32 and 56-60 differed among three herds. In addition, Zobel *et al*. (28) reported that there was a difference in the occurrence of late embryonic mortality between two types of farms, namely, pasture-based and intensive, indicating that farm management might play a significant role in the late embryonic mortality of dairy cows. These discrepancies in the incidence of late embryonic mortality among different studies and herds might be associated with differences in facilities, bunk space, farm management (including nutrition, reproduction, and health controls), environment (climate and weather), periods of embryonic mortality detection, and other factors.

Our findings on the higher incidence rate for late embryonic mortality in cows inseminated during May-July might be associated with high temperature and humidity during the summer season (June-August) in Korea. The higher incidence rate during these months may in part be explained by the fact that embryo implantation occurs on gestational days 21 to 30 in the cow (10). Another study (9) reported that the likelihood of pregnancy loss increases by a factor of 1.05 for each additional unit of the mean temperature-humidity index (THI) from gestational days 21 to 30. These findings support our results. In addition, Santolaria *et al*. (22) reported that a one-unit increase in the cumulative number of hours with a maximum THI > 85 (temperatures higher than 37°C with a relative humidity higher than 40%) during gestational days 11 to 20 results in a 1.57-fold increase in pregnancy loss. On the other hand, in a study that analyzed conceptus-maternal interactions by measuring plasma pregnancy-associated glycoprotein concentrations, the development of a healthy placenta was strongly linked to gestations occurring from December to February (25). These findings also support our results, which showed that the frequency of late embryonic mortality was lower in cows inseminated from November to January. Due to the negative effects of heat stress on pregnancy loss during hot temperatures, farm management should include fans, water sprinklers and shaded areas for cows as suggested by Santolaria *et al*. (22).

Cows inseminated after estrus following $PGF_{2\alpha}$ treatment had a higher risk, whereas cows inseminated following the Ovsynch program tended to have a lower risk of late embryonic mortality than cows inseminated following natural estrus. Our findings are supported in part by a previous study which showed that embryo survival decreases when estrus is induced by sequential injections of $PGF_{2\alpha}$ and the ovulatory follicle develops in the presence of a low progesterone environment (23). Inconsistent with our findings, however, are results showing similar rates of embryonic and fetal mortality between timed AI protocols, such as the Ovsynch and Heatsynch programs, and insemination upon the detection of estrus (23). Moreover, it has been reported that cows inseminated following spontaneous estrus may have a lower rate of embryonic death than those bred following timed AI (19). The reason for this discrepancy between studies is not clear, and additional studies are needed to define the relationship between synchronization protocol and embryo survival.

Our study showed that postpartum disease (including retained placenta, abomasal displacement, endometritis, and ovarian cyst) tended to be a risk factor for late embryonic mortality in dairy cows. A previous study reported that endometritis increased the risk of pregnancy loss between gestational days 30 and 65 days (20). Other studies also demonstrated that pyometra, retained placenta, and mastitis are important risk factors for pregnancy loss (11,15). Results from the present and other studies indicate that prevention of periparturient and postpartum diseases might eliminate late embryonic mortality and improve reproductive performance in dairy cows.

In summary, logistic regression analysis revealed that herd size (herd size < 50 lactating cows), AI month (May-July), $PGF_{2\alpha}$-induced synchronization protocol, and postpartum disease were important risk factors for late embryonic mortality in dairy herds. Therefore, improvement of herd management by including facilities that reduce heat stress and incorporating effective reproductive program (Ovsynch) and health control might help to prevent late embryonic mortality in dairy cows.

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