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Risk factors for repeat breeder dairy cows and their impacts on reproductive performance

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Abstract

The objective of this study was to identify risk factors for repeat breeder dairy cows and their impact on reproductive performance. The characteristics of 1,504 cows, including their peripartum health, nutrition, production, and reproduction, were collected. Cows with metabolic disorder were significantly more likely (odds ratio [OR], 2.47; $p < 0.001$) and cows with clinical endometritis tended to be more likely (OR, 1.35; $p < 0.1$) to become repeat breeders than cows without metabolic disorder and clinical endometritis, respectively. Cows initially inseminated > 80 days after calving were less likely (OR, 0.78; $p < 0.05$) to become repeat breeders than cows initially inseminated ≤ 80 days after calving. As a result, repeat breeding in dairy cows resulted in a 90 day longer ($p < 0.0001$) mean interval from calving to pregnancy. In conclusion, postpartum metabolic disorder, clinical endometritis, and a short interval from calving to initial insemination were risk factors for repeat breeder dairy cows, resulting in a severely impaired reproductive performance.

Keywords: repeat breeder cow; risk factors; postpartum disorder; breeding time; reproductive performance

Introduction

A repeat breeder is defined as a cow that fails to become pregnant after 3 or more consecutive inseminations within the same lactation period without any detectable abnormalities in the genital tract and with apparently normal estrous cycles [1–3]. This condition markedly reduces reproductive performance due to the increased number of inseminations and longer calving intervals, thereby increasing culling and replacement costs in dairy cows [4,5]. Because the causes remain unknown and/or may be covered by other symptoms, it is very difficult to determine methods to overcome this disorder. Likewise, methods of prevention and treatment have not yet been established, except for the administration of hormones, including gonadotropin-releasing hormone (GnRH) and progesterone, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), or combinations of these agents [3,6,7], or the use of embryo transfer techniques [8,9]. Thus, the identification of risk factors for repeat breeder cows might help to design effective measures to prevent and/or treat this reproductive disorder.

Previous studies have suggested that alterations in hormone concentrations, including high estradiol concentrations at estrus and reduced luteinizing hormone (LH) concentrations before peak LH or subluteal progesterone concentrations [10,11], are physiological factors associated with repeat breeder cows. Other phys-

iological factors include impaired oocyte competence [12,13], ovulation defects [11], early embryonic death [14], and an altered or impaired uterine environment [15,16]. In addition, peri- and postpartum diseases, such as dystocia, stillbirth, metritis, cystic ovarian disease, subclinical endometritis [17,18], delayed time to first estrus after calving [19], and short time from calving to initial insemination [20], have also been reported to be factors associated with repeat breeder cows.

Despite studies reporting risk factors for repeat breeder cows, the factors identified have been inconsistent, as they may be affected by management practices, such as intensive or extensive production systems; the productive and/or reproductive capacities of individual animals or herds; and/or geographic or regional characteristics. Despite poor housing conditions, such as limited space per cow and inadequate feeding, milk production per cow has increased annually in Korea using an intensive production system. These dairy management practices make it easier for cows to become repeat breeders, reducing their reproductive performance. However, the incidence of repeat breeder dairy cows in Korea has not been determined in recent years. Thus, the identification of risk factors for repeat breeder dairy cows maintained under intensive productive management and providing a high yield of milk may provide valuable information on methods to reduce the incidence of repeat breeder dairy cows. Therefore, the present study was designed to identify risk factors for repeat breeder dairy cows by analysing their peripartum disorders, nutrition, milk production, and reproduction, collected during periodic evaluations of herd health and reproduction.

Materials and Methods

Animals and health and reproductive management

This study was conducted on 12 dairy farms in Chungcheong Province, Korea. Each farm contained between 50 and 250 cows, which were maintained in loose housing systems and fed total mixed rations, and milked twice daily. The mean annual milk yields per cow on these farms ranged from 9,195 to 11,590 kg. Animal experiments were performed with the approval of the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (CBNUA-1299-19-02).

All cows in the participating farms underwent reproductive health checks every 2 to 4 weeks. These included an examination of their ovarian structures (follicle and corpus luteum [CL]) and uterus by transrectal palpation and ultrasonography. At the time of these examinations, the body condition scores (BCS) of these cows were evaluated using a previously developed visual technique [21].

Peri- and postpartum disorders were defined as described [22–24]. Dystocia was defined as calving requiring assistance. Retained placenta was defined as the retention of the fetal membranes for longer than 24 hours. Septicemic metritis was defined as a fever (rectal temperature ≥ 39.5°C) and a watery, fetid, red-brown uterine discharge. Ketosis was diagnosed as the occurrence of anorexia and depression, and the odor of acetone on the breath. Milk fever was diagnosed as the occurrence of nervousness, weakness, and recumbency after calving. Abomasal displacement was diagnosed by abdominal auscultation. Clinical endometritis was diagnosed as the occurrence of a mucopurulent uterine discharge and by transrectal palpation and ultrasonography. Cows diagnosed with septicemic metritis were subcutaneously administered antibiotics and supportive medication for 3 to 5 days. Ketotic cows were treated with propylene glycol, dextrose, or glucocorticoids, and those diagnosed with milk fever were treated with a calcium preparation. Abomasal displacement was corrected surgically by right or left flank laparotomy. Cows with clinical endometritis and CL were treated with $PGF_{2\alpha}$, whereas cows with clinical endometritis but without CL were administered a single intrauterine infusion of 2% povidone-iodine solution, and were re-treated if necessary.

The voluntary waiting period from calving to the first artificial insemination (AI) was 40 days. In addition to estrus detection, herd reproductive programs, such as Ovsynch or 2 modified presynchronization-Ovsynch (modified Presynch-Ovsynch or Double-Ovsynch) were employed. For Ovsynch, cows were administered GnRH on day 65 (calving = day 0), $PGF_{2\alpha}$ on day 72, and GnRH 56 hours later, followed by timed AI 16 hours later. For modified presynchronization-Ovsynch programs, cows were administered $PGF_{2\alpha}$ (Presynch-Ovsynch) or GnRH (Double-Ovsynch) on day 45, followed by $PGF_{2\alpha}$ on day 55 and GnRH on day 58, followed by Ovsynch 7 days later. Pregnancy was evaluated 31 and 48 days after AI. If cows did not conceive following the first AI, those that exhibited natural estrus were again inseminated according to the am-pm rule. Cows confirmed as not being pregnant by ultrasonography were resynchronized using Ovsynch, with the resynchronization programs continued until the cows became pregnant. Reproductive performance data were collected for a minimum of 210 days postpartum or until pregnancy or culling.

Data collection and statistical analyses

The characteristics of 1,504 dairy cows (574 primiparous and 930 multiparous) on 12 Holstein dairy farms were recorded.

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Factors collected included detailed information regarding parity, the occurrence of peri- or postpartum disorders (e.g., dystocia, retained placenta, metabolic disorder including ketosis, milk fever or abomasal displacement, septicemic metritis, and clinical endometritis), BCS, milk yield during the first 3 months, and dates of previous calving, AI, and confirmation of pregnancy.

Data are expressed as the mean ± standard error of the mean (SEM). For statistical analysis, cows were categorized as either primiparous or multiparous, by herd size (≤ 60 or > 60 lactating cows), by BCS loss between calving and 30 days after calving (no or yes), by the interval between calving and first insemination (≤ 80 or > 80 days), and by mean milk yield during the first 3 months postpartum (≤ 40 or > 40 kg). All statistical analyses were performed using SAS ver. 9.4 (SAS Institute, USA).

The risk of repeat breeder cows was analysed by logistic regression using the LOGISTIC procedure. Factors in the logistic regression model included herd size, parity, dystocia, retained placenta, metabolic disorder, septicemic metritis, clinical endometritis, BCS loss between calving and 30 days after calving, interval (days) between calving and first insemination, milk yield, and interactions among these variables. Backward stepwise regression was used in all the models, with elimination performed based on the Wald statistic criterion when $p > 0.10$. Odds ratios (OR) and 95% confidence intervals were determined using logistic regression.

A Cox's proportional hazard model and the PHREG procedure were used to compare the probability of pregnancy by 210 days postpartum between repeat breeders and non-repeat breeders. This yielded an estimate of the likelihood of a cow being pregnant at a given time. The time variable used in this model was the interval in days between calving and pregnancy. Cows that died, were sold, or were not pregnant by 210 days postpartum were not included in the analysis. The Cox model included herd size, parity, dystocia, retained placenta, metabolic disorder, septicemic metritis, clinical endometritis, BCS loss between calving and 30 days after calving, interval between calving and first insemination, milk yield, and interactions among these variables. Proportional hazards were determined based on interactions between explanatory variables and time, and by evaluating Kaplan-Meier curves. The median and mean number of days to pregnancy was determined by survival analysis in the Kaplan-Meier model using the LIFETEST procedure within the SAS software. A survival plot was generated using the Survival option of MedCalc software (ver. 11.1; MedCalc Software, Belgium). A p-value ≤ 0.05 was considered statistically significant, whereas a p-value > 0.05 but < 0.1 was considered to indicate a trend.

Results

Reproductive and productive characteristics of the cows

The 1,504 cows included in this study had a mean ± SEM parity of 2.23 ± 0.04. The incidence rates of dystocia, retained placenta, metabolic disorder, septicemic metritis, and clinical endometritis were 5.3%, 10.1%, 0.9%, 4.2%, and 11.2%, respectively. The mean BCSs at calving and 30 days after calving were 3.26 ± 0.01 and 3.12 ± 0.01, respectively, and the mean interval from calving to first insemination was 76.7 ± 0.4 days. Mean milk yields 30, 60, and 90 days after calving were 39.8 ± 0.2 kg, 41.5 ± 0.2 kg, and 40.4 ± 0.2 kg, respectively, with an average of 40.6 ± 0.2 kg. The pregnancy rates per AI after the first, second, and third inseminations were 35.0%, 27.7%, and 38.3%, respectively.

The overall mean incidence rate of repeat breeder dairy cows was 30.0%. Table 1 shows the overall descriptive statistics for the incidence of repeat breeder cows.

Risk factors for repeat breeder dairy cows

Table 2 shows the risk factors for repeat breeder dairy cows. The occurrences of a metabolic disorder and clinical endometritis, as well as a shorter time between calving and first insemination, were found to be risk factors for repeat breeder dairy cows. Cows that had metabolic disorder were significantly more likely (OR, 2.47; $p < 0.001$) to become repeat breeders than cows without metabolic disorder. In addition, cows that had clinical endometritis tended to be more likely (OR, 1.35; $p < 0.1$) to become repeat breeders than cows without endometritis. Furthermore, cows initially inseminated > 80 days after calving were significantly less likely (OR, 0.78; $p < 0.05$) to become repeat breeders than cows initially inseminated ≤ 80 days after calving. By contrast, herd size, parity, dystocia, retained placenta, septicemic metritis, BCS loss between calving and 30 days postpartum, and milk yield were not risk factors for repeat breeder dairy cows ($p > 0.1$).

The impact of repeat breeding on reproductive outcomes in dairy cows

Table 3 shows the factors that affected the likelihood of pregnancy by 210 days after calving. Repeat breeder cows were less likely to become pregnant (hazard ratio, 0.08; $p < 0.0001$) than non-repeat breeder cows, significantly extending ($p < 0.0001$) the mean interval between calving and pregnancy by 90 days

Table 1. Factors associated with the incidence of repeat breeder dairy cows

Variable	Level	Repeat breeder+	Repeat breeder-
Dystocia	No	420	1,005
	Yes	31	48
Retained placenta	No	405	947
	Yes	46	106
Metabolic disorder	No	419	1,022
	Yes	32	31
Septicemic metritis	No	446	1,044
	Yes	5	9
Clinical endometritis	No	390	946
	Yes	61	107
Herd size	≤ 60 lactating cows	167	410
	> 60 lactating cows	284	643
Parity	Primiparous	180	394
	Multiparous	271	659
Body condition scores loss between calving and 30 days postpartum	No	224	556
	Yes	227	497
Interval between calving and first insemination	≤ 80 days	306	663
	> 80 days	145	390
Milk yield during the first 3 months postpartum	≤ 40 kg	220	501
	> 40 kg	231	552

Table 2. Odds ratios for variables included in the logistic regression model for the risk of repeat breeder dairy cows

Variable	Odds ratio	95% confidence interval	*p*-value
Metabolic disorder*			
No	Reference		
Yes	2.47	1.480–4.107	< 0.001
Clinical endometritis			
No	Reference		
Yes	1.35	0.962–1.903	< 0.1
Interval between calving and first insemination			
≤ 80 days	Reference		
> 80 days	0.78	0.618–0.992	< 0.05
Dystocia			> 0.1
Retained placenta			> 0.1
Septicemic metritis			> 0.1
Herd size†			> 0.1
Parity			> 0.1
Body condition score loss‡			> 0.1
Milk yield§			> 0.1

*Metabolic disorders included ketosis, milk fever, and abomasal displacement.
†Herd size was categorized as ≤ 60 or > 60 lactating cows.
‡Body condition score loss between calving and 30 days after calving was categorized as no or yes.
§Milk yield during the first 3 months postpartum was categorized as ≤ 40 or > 40 kg.

(Fig. 1). In addition, herd size, dystocia, metabolic disorder, interval between calving and first insemination, and milk yield affected the likelihood of pregnancy by 210 days after calving.

Discussion

This retrospective study identified risk factors for repeat breeder dairy cows and their impact on reproductive perfor-

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Table 3. Factors affecting the probability of pregnancy by 210 days postpartum, identified using the PHREG procedure

Variable	Hazard ratio	95% confidence interval	*p*-value
Repeat breeder			
No	Reference		
Yes	0.08	0.062–0.090	< 0.0001
Herd size			
≤ 60 lactating cows	Reference		
> 60 lactating cows	1.26	1.117–1.420	< 0.001
Dystocia			
No	Reference		
Yes	0.7	0.527–0.936	< 0.05
Metabolic disorder*			
No	Reference		
Yes	0.52	0.367–0.739	< 0.001
Interval between calving and first insemination			
≤ 80 days	Reference		
> 80 days	0.6	0.526–0.674	< 0.0001
Milk yield			
≤ 40 kg	Reference		
> 40 kg	0.85	0.725–0.996	< 0.05
Retained placenta			> 0.1
Septicemic metritis			> 0.1
Clinical endometritis			> 0.1
Parity			> 0.1
Body condition score loss†			> 0.1

*Metabolic disorders included ketosis, milk fever, and abomasal displacement.
†Body condition score loss between calving and 30 days after calving was categorized as no or yes.

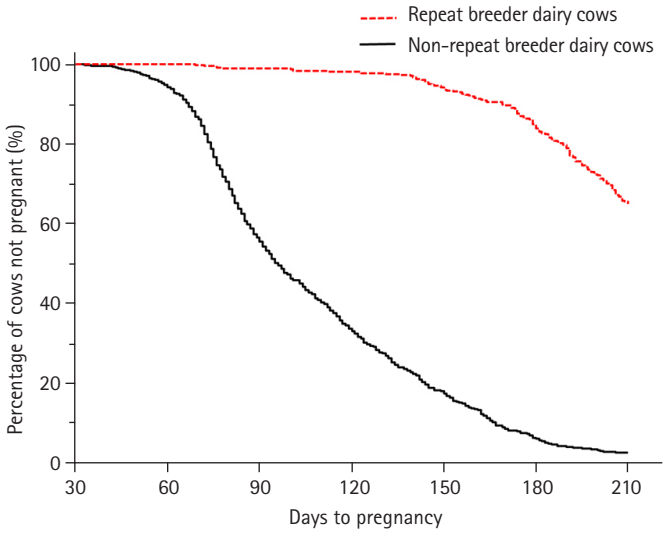

Fig. 1. Survival curves for the interval between calving and pregnancy in repeat breeder (n = 451) and non-repeat breeder (n = 1,053) dairy cows. The probability of pregnancy by 210 days postpartum was significantly lower ($p < 0.0001$) in repeat breeder (hazard ratio, 0.08) than in non-repeat breeder dairy cows. The mean intervals between calving and pregnancy were 198.2 ± 1.1 days and 108.0 ± 1.2 days in repeat breeder and non-repeat breeder dairy cows, respectively ($p < 0.0001$).

mance. Cows that had postpartum metabolic disorder or clinical endometritis were more likely to become repeat breeders than those that did not, whereas cows that underwent initial AI > 80 days after calving were less likely to become repeat breeders than cows that underwent initial AI ≤ 80 days after calving. Reproductive performance was more impaired in repeat breeder than in non-repeat breeder cows, with the mean interval between calving and pregnancy extended by 90 days.

The incidence rate (30.0%) of repeat breeder dairy cows in the present study was higher than previously reported rates (14.0% to 24.0%) [4,5,20]. Although the reasons for the higher incidence rate in the present study remain unknown, unfavorable farm conditions, such as their facilities, available space, and management system, and differences in the productive and/or reproductive characteristics of the animals, may be associated with incidence rate. These findings suggest the need for more careful attention to the higher incidence of repeat breeder cows under an intensive productive system, and the need for suitable strategies to prevent this disorder.

The present study found that metabolic disorders and clinical endometritis were risk factors for repeat breeder dairy cows. Al-

though metabolic disorders have not been previously reported to be a risk factor for repeat breeder cows, postpartum metabolic disorders, including abomasal displacement and milk fever, were found to adversely affect subsequent reproductive performance in dairy cows [25,26]. Because a large proportion of cows with metabolic disorder in the present study had abomasal displacement, the sequela of the abomasal displacement might be associated with the increased incidence of repeat breeders. Cows with abomasal displacement were found to have higher neutrophil counts in the uterus, increasing the risk of concurrent clinical endometritis, which was associated with impaired reproductive performance [25].

Our finding, that clinical endometritis was a risk factor for repeat breeder cows, is supported by results showing that cytological endometritis not only significantly decreased reproductive performance but also increased the incidence of repeat breeder dairy cows [18]. Although the exact mechanism responsible for the association between endometritis and repeat breeders has not been determined, endometritis has been reported to suppress the releasing of GnRH and LH, to inhibit ovulation of a dominant follicle, and/or to impair the uterine environment [16,27]. However, other studies showed that cytological endometritis was not significantly associated with the incidence of repeat breeder cows [2,28]. These differences between studies using uterine cytology may be due to differences in thresholds (the percentage of neutrophils among total endometrial cells) and/or the time of collection of the uterine samples (i.e., during the voluntary waiting period or before AI) used to define cytological endometritis. By contrast, long-term infertility in repeat breeder cows may be associated with alterations in endometrial function, induced by changes in endometrial gene expression [15,29].

Our finding, that cows with a longer interval between calving and first insemination were less likely to become repeat breeders than those that had a shorter interval, is consistent with a previous study [20]. The mechanism responsible for the association of a shorter interval between calving and first insemination and a high risk for repeat breeder cows remains unclear. However, a longer interval between calving and first insemination may be associated with improved uterine health, reduced systemic inflammation, and more time to resume ovarian cyclicity [30].

The present study also found that reproductive performance was significantly lower in repeat breeder than in non-repeat breeder cows, with cumulative pregnancy rates by 210 days postpartum of 34.8% and 97.0%, respectively (data not shown). This resulted in a 90 day longer mean interval between calving and pregnancy in repeat breeder dairy cows. The cumulative pregnancy rate by 210 days postpartum in repeat breeder cows in the present study was similar to findings showing that only 31.4% of repeat breeder dairy cows conceived within 210 days postpartum [20]. Another study showed that the calving-to-conception interval was 187 days longer in repeat than in non-repeat breeder cows [31]. Moreover, the OR of pregnancy in repeat compared with non-repeat breeder cows was 0.73 [5]. The higher incidence of repeat breeder cows increased the interval from calving to conception and increased culling in dairy herds, leading to severe economic losses [4,17,32].

A marked alteration in BCS during the postpartum period was found to adversely affect reproductive performance [33,34], whereas the effect of milk yield during the early lactation period on reproductive performance remains unclear [35,36]. The present study found that BCS loss did not affect the likelihood of pregnancy by 210 days postpartum, whereas a higher milk yield had a negative effect on the likelihood of pregnancy, suggesting that milk yield during the early lactation period affected long-term but not short-term fertility, as milk yield was not a risk factor for repeat breeder cows.

In summary, the present study has identified the risk factors for repeat breeder dairy cows and their impact on reproductive performance. Metabolic disorder, clinical endometritis, and a shorter interval between calving and first insemination were associated with the likelihood of becoming repeat breeders, severely impairing reproductive performance. An appropriate health strategy to prevent postpartum disorders, especially metabolic disorder and clinical endometritis, may reduce the likelihood of becoming repeat breeders. In addition, initial insemination at the proper time, not too early during the postpartum period, might reduce the incidence of repeat breeder dairy cows, enhancing their reproductive performance.

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