

# Effects of Twin Birth on the Occurrence of Postpartum Disorders, Culling and Reproductive Performance, and its Risk Factors in Dairy Cows

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Abstract : This study determined the effects of twin birth on subsequent postpartum disorders, culling and reproductive performance, and its risk factors in dairy cows. Detailed data related to reproduction, health, and calving events, including dates of artificial insemination (AI) and calving, hormonal treatment prior to conception, cow parity, twin birth, retained placenta, metabolic disorders, and endometritis were collected from 1,717 individual calvings on 9 dairy farms. The incidence rate of twin birth was 3.4%. Gestation length was shorter in cows with twin birth  $(270.6 \pm 2.0)$ days) than cows giving birth to singletons (279.5  $\pm$  0.2 days, P < 0.01). The incidence of retained placenta (47.5 vs. 16.0%), metabolic disorders (18.6 vs. 3.8%) endometritis (62.7 vs. 28.2%), and culling rate (32.2 vs. 16.5%) was greater in cows with twin birth than in cows giving birth to singletons, respectively (P < 0.01). The hazard of pregnancy (adjusted hazard ratio, AHR) by 210 days in milk analyzed using Cox proportional hazards model was affected by calving season, in that the rate of pregnancy for cows calved during spring was less than for cows calved during winter (AHR = 0.80; P = 0.01); this was also true for endometritis (AHR = 0.46, P < 0.01). AHR was not affected by twin birth. Logistic analysis demonstrated that increasing the cow parity (P < 0.01) and hormonal treatment prior to conception (adjusted odds ratio, AOR = 1.84, P < 0.05) increased the risk of twin birth. These results demonstrate that twin births are responsible for severe economic losses through the increased occurrence of postpartum disorders and culling; thus, appropriate management for the deleterious impact of twin birth, and/or reducing the risk factors by herd control regarding cow parity and the use of reproductive hormones prior to conception might be necessary.

Key words: twin birth, postpartum disorders, culling, dairy cows.

#### Introduction

Twin birth is regarded as an undesirable condition in dairy cattle management due to reduced profitability, which has been estimated to amount to up to \$108 to \$119 per twin birth through the negative impacts on the dams and calves born (2,10). The incidence of twin birth has been reported in a range between 2.4 to 6.9% worldwide (1,6,10,13,18,22,23,26). An unexpected increase in the incidence of twin birth over time has become a growing concern in dairy cattle management (17,18,26). Presumably, current dairy management strategies directed to increase milk yield may contribute to an increase in the frequency of double ovulation and twin birth in dairy cows (11,17,18,22,28).

In dairy management, twin birth can cause detrimental effects related to health, production, and reproduction on dams and calves born as twins. Cows calving twins may evidence a greater incidence of abortion (22), dystocia (3,14), retained placenta (3,10,22), uterine diseases (3,8,10) and metabolic disorders (20,22), as well as lower calf survival (6) than cows who calve singletons. Moreover, the consequent

negative impact on the cows calving twin births may include poorer reproductive performance (10,14,22) as well as increased culling (3,4,7,10,22) due to periparturient complications and/ or fertility issues. Under the consideration of the negative impact by twin birth on the dams and calves born, the determination of risk factors for the condition might prove worthwhile as part of a strategy to avoid the severe losses caused by twin births.

The risk factors for twin birth are multi-factorial, and include genetic, physiologic and environmental components; heritability (17), breed (24), cow parity (3,13,15,18,25,26), season of conception /calving (13,22,25,26), milk production (18,22) and hormonal treatment prior to conception (18,22). Identifying the risk factors for twin birth and its impact on the subsequent animal health and fertility from different continents in diverse environments may enable veterinarians and farmers to obtain profitable prevention and management practices. Therefore, the principal objective of this study was to determine the impact of twin birth on subsequent postpartum disorders, culling, and reproductive performance and its risk factors in dairy herds.

## **Materials and Methods**

#### Herds and animal management

This study was performed on 9 dairy farms located in the

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Chungbuk Province. All herds contained 50 or more cows, and all the cows were milked twice daily, maintained in free-stall facilities, and fed a total mixed ration. The herds were maintained under regular reproductive health checkups every 2 to 4 weeks by veterinarians at the College of Veterinary Medicine at Chungbuk National University. The regular reproductive health checkup included the diagnosis, prevention, and treatment of reproductive disorders, pregnancy diagnosis using ultrasonography, and measurement of body condition scores. The voluntary waiting period for rebreeding was 40 days. Cows were observed for estrus twice daily and artificial insemination (AI) was carried out in accordance with the a.m.-p.m. rule. In addition, a herd reproductive management program including synchronization of estrus using PGF<sub>2a</sub> (Lutalyse<sup>®</sup>, Phamacia & Upjohn, Belgium), or ovulation using PGF<sub>2 $\alpha$ </sub> and GnRH (Fertagyl, Intervet, Netherland) with or without internal drug-release device containing 1.9 g of progesterone (CIDR<sup>TM</sup>, InterAg, Hamilton, New Zealand) was administered. Pregnancy was diagnosed rectally 40 to 50 days after AI by both ultrasonography (Sonoace 600 with 5.0 MHz linear-array transducer; Medison Co., Ltd., Seoul, Korea) and manual palpation.

#### Data collection and statistical analysis

Detailed health, reproduction and other environmental records from 1,717 individual calvings on 9 dairy farms were collected. Dates of AI, conception and calving, hormonal treatment prior to conception (synchronization of estrus/ovulation), cow parity, twin birth, retained placenta, metabolic disorders including abomasal displacement, milk fever and ketosis, and endometritis were included in the dataset. For statistical analysis in this study, cow parity was categorized into parity values of 1, 2, 3 or  $4 \le$ , and calving /conception season was grouped as spring (March to May), summer (June to August), fall (September to November) and winter (December to February).

Statistical analysis of gestation length was carried out using the general linear models procedure. The model included cow parity, calving season, and twin birth. Categorical data, which included the incidence of retained placenta, metabolic disorders and endometritis, and culling were compared via Chisquare test between cows with twin birth and cows with a singleton. Reproductive performance was evaluated as the interval from calving to conception (days). The effect of twin birth on the hazard of pregnancy (adjusted hazard ratio, AHR) by 210 days in milk (DIM) was analyzed by Cox's proportional hazard model using the PHREG procedure. This analysis estimated the hazard of a cow being pregnant at a given time. Thus, it measured the rate of pregnancy. The time variable used in the model was the interval between calving and pregnancy, which was detected between 40 and 50 days after AI. Cows that were not pregnant by 210 DIM, or were culled or died, were censored. The variables used in the Cox model were farm, cow parity, calving season, twin birth and endometritis. Proportionality was evaluated via Kaplan-Meier curves. The median days to pregnancy were obtained via survival analysis from the Kaplan-Meier model using the LIFETEST procedure. The survival plot was generated using the survival option of MedCalc version 11.1 for Windows (MedCalc Software, Mariakerke, Belgium). The risk factors for twin birth were analyzed via logistic regression using the LOGISTIC procedure. The model included farm, cow parity, season of conception, hormonal treatment prior to conception, and interactions between cow parity and hormonal treatment, and season of conception and hormonal treatment. In the model, a backward stepwise regression was employed and the elimination was carried out in accordance with Wald's statistical criterion, in which P > 0.11. The adjusted odds ratio (AOR) and 95% confidence interval (CI) were generated during the logistic regression. All statistical analyses were carried out using SAS software (SAS version 9.1, SAS Inst. Inc., Cary, NC). Differences with P < 0.05 were considered significant.

### Results

The incidence rate of twin birth was 3.4% in a population of 1,717 calvings. Gestation length was shorter (P < 0.01) in cows with twin birth (270.6  $\pm$  2.0 days) than cows with a singleton (279.5  $\pm$  0.2 days). The effects of twin birth on the postpartum metabolic and reproductive disorders are shown in Fig 1. The incidence of retained placenta (47.5 vs. 16.0%), metabolic disorders (18.6 vs. 3.8%) and endometritis (62.7 vs. 28.2%) was greater in cows with twin birth than cows with a singleton, respectively (P < 0.01). Moreover, the culling rate due to postpartum complications and reproductive failure was also greater (P < 0.01) in cows with twin birth (32.2%) than cows with a singleton (16.5%). The Cox proportional hazards model showed that the hazard of pregnancy by 210 DIM was affected by calving season and endometritis, not by twin birth. The rate of pregnancy for cows calved during spring was less than for cows calved during winter (AHR = 0.80; P = 0.01), which resulted in extended median interval to pregnancy (165 vs. 133 days). Cows with endometritis also reduced the pregnancy rate (AHR = 0.46, P < 0.01) than cows without endometritis, which resulted in extended median interval to pregnancy

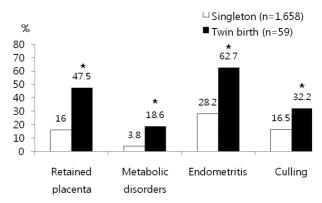
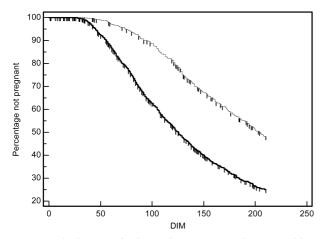


Fig 1. The effects of twin birth on the occurrence of the postpartum metabolic and reproductive disorders, and culling in dairy cows. \*represents significant differences between cows with a singleton and cows with twin birth (P < 0.01).



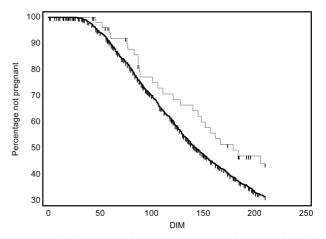
**Fig 2.** Survival curves for interval to pregnancy in cows with or without endometritis. Cows with endometritis (n = 504; dashed line) reduced (adjusted hazard ratio = 0.51; CI = 0.45-0.58, P < 0.01) the pregnancy rate by 210 DIM relative to cows without endometritis (n = 1,210; solid line). Median day to pregnancy was 204 days for cows with endometritis and 125 days for cows without endometritis, respectively. Tick marks in the curves represent censored cows.

(204 vs. 125 days, Fig 2). Kaplan-Meier curves also showed that twin birth tended to decrease (P = 0.08) the rate of pregnancy, resulting in median intervals to pregnancy of 179 and 156 days for cows with twin birth and cows with a singleton (Fig 3).

Our logistic procedure identified cow parity and hormonal treatment prior to conception as risk factors for twin birth (Table 1); the occurrence of twin birth increased with increasing parity (P < 0.01). Additionally, cows with hormonal treatment (synchronization of estrus / ovulation) prior to conception had an increased likelihood of twin birth (AOR = 1.84, P < 0.05) as compared to cows without hormonal treatment. However, farm and season of conception had no effect.

### Discussion

In accordance with the results of previous studies, twin birth was associated with greater incidence of retained placenta, metabolic disorders, and endometritis, even for higher culling rates. Important risk factors for twin birth were identified as



**Fig 3.** Survival curves for interval to pregnancy in cows with twin birth or cows with a singleton. Pregnancy rate by 210 DIM did not differ (adjusted hazard ratio = 0.74; CI = 0.53-1.03, P = 0.08) between cows with twins birth (n = 59; dashed line) and cows with a singleton (n = 1,658; solid line). Median day to pregnancy was 179 days for cows with twin birth and 143 days for cows with a singleton, respectively. Tick marks in the curves represent censored cows.

cow parity and hormonal treatment prior to conception. The establishment of a proper treatment regimen for postpartum complications following the incidence of twin birth and/ or prevention of twin birth by reducing its risk factors by herd control regarding cow parity and the use of reproductive hormone prior to conception might be necessary to ameliorate economic losses.

The twin-birth incidence rate of 3.4% remained within the previously reported ranges of 2.4 to 6.9% (1,6,10,13,18,22,23, 26), although this represented a comparatively lower rate within the range. Gestation length was shortened by 9 days in cows with twin-birth as compared to singletons in this study; this result is consistent with other reports (3,5,9,25,27).

The increased incidence of postpartum disorders such as retained placenta, endometritis, and metabolic disorders following twin birth observed in the present study is in overall agreement with the results of other previous studies (3,8,10, 18,22), which might be caused by shortened gestation length, difficult calving, and other nutritional, physical or physiological stress due to twin birth. Furthermore, the culling rate due to

Table 1. Adjusted odds ratios (AOR) of variables included in the final logistic regression model for risk of twin birth in dairy cows

Variable	Level	Twin birth, % (n/n)	AOR	95% CI	P - value
Cow parity	1	0.7 (4/551)	Reference		
	2	2.4 (11/456)	2.98	[0.93, 9.51]	0.07
	3	5.4 (17/318)	6.64	[2.18, 20.22]	< 0.01
	4 ≤	6.9 (27/392)	8.65	[2.96, 25.33]	< 0.01
Hormonal treatment*	No	2.8 (42/1,489)	Reference		
	Yes	7.5 (17/228)	1.84	[1.01, 3.33]	< 0.05

\*Hormonal treatment for synchronization of estrus (PGF<sub>2 $\alpha$ </sub>) or ovulation (PGF<sub>2 $\alpha$ </sub> and GnRH with or without CIDR) prior to conception

postpartum disorders and reproductive failure was also greater in cows with twin birth than singletons in this study; this result is also similar to that seen in previous reports (4,7,10).

In the present study, Cox's proportional hazard model using the PHREG procedure showed that the calving-to-conception interval was not affected by twin birth, whereas calving season and endometritis significantly affected the interval. One study demonstrated that the calving-to-conception interval did not differ between twin-calving and single-calving cows (3), which was consistent with our result. However, several other studies have demonstrated the extended interval from calving to conception (4,10,14) attributable to twin birth. The discrepancy between our study and other studies regarding the effect of twin birth on the subsequent reproductive performance may be attributable to the small scale of animals in our study, resulting in lower statistical power. Our observation using the Kaplan-Meier curves that twin birth tended to reduce the rate of pregnancy might also be associated with the small samples. On the other hand, the effect of calving season in which the rate of pregnancy for cows calved during spring was less than for cows calved during winter in this study has also been demonstrated in another report (14), which may be attributable to delayed rebreeding during the subsequent hot summer season. Our observation of the adverse effect of endometritis on reproductive performance has also been demonstrated in previous studies (19,21). Considering the results observed in our and previous studies regarding the deleterious impact of twin birth, efficient strategies to avoid or reduce the negative effects of twin birth should be established as a part of dairy practices.

Import risk factors for twin birth were cow parity and hormonal treatment (synchronization of estrus / ovulation) prior to conception in this study. The observed increase in the incidence of twin birth with increasing parity is consistent with the results of other studies (3,5,10,18). Although the effect of cow parity on the increased incidence of twin birth has yet to be clarified, it might be explained in part by the finding that the incidence of double ovulation increases with parity in lactating dairy cows (11). Additionally, milk production is confounded with parity, and might explain the changes associated with cow parity (12). Interestingly, the use of reproductive hormones for the synchronization of estrus or ovulation prior to conception increased the risk of twin birth in this study, which were similar to the previous studies (18,22). The mechanism underlying the effects of reproductive hormones prior to conception on the increased incidence of twin birth has yet to be definitively elucidated, but the use of hormones prior to conception might disrupt the physiological regulation of ovulation, resulting in the ovulation of more than one follicle at the same time. The season of conception was not associated with the incidence of twin birth, which is consistent with the results of other studies (10,18). Contrary to our results, however, several other reports have demonstrated the effects of season of conception on the incidence of twin birth. An increased incidence of twin birth was noted in cows calved during April to July (spring and early summer) that would have been conceived during August to November (late summer and fall) (5,15,25,26). Similarly, another study showed that conception during September to December increased the incidence of twin birth (13). It has been speculated that the tendency toward the increase in twin birth in cows conceived during late summer until fall might be explained by a reduction in early embryonic loss attributable to reduced heat stress during a time of increased double ovulations and conception of twins (26). Additionally, photoperiod and/or increased plane of nutrition were also found to affect the seasonal variation in twin birth (16,22). To cope with the negative effects of twin birth, these potential risk factors must be avoided or ameliorated by appropriate dairy herd management. Besides, the early identification of twins via ultrasonography and removal of an fetus, or earlier dry-off and feeding of a transition diet, as well as careful assistance with the calving of cows carrying twins may also help to reduce the economic losses due to twin birth (3,12).

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# 젖소에서 쌍태분만이 산후질병 발생, 도태 및 번식능력에 미치는 영향과 쌍태 위험 요인

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**요 약**: 젖소에서 쌍태분만이 이후의 산후질병 발생, 도태, 번식능력에 대한 영향과 쌍태분만에 대한 위험요인을 조사 하였다. 9개 목장 1,717 분만축의 번식, 위생 및 분만관련 상세 자료가 분석에 이용되었다. 쌍태 분만율은 3.4%였으며, 임신기간은 쌍태분만 시가 단태분만 시에 비해 9일 단축되었다(270.6±2.0 vs. 279.5±0.2일, P<0.01). 쌍태분만 시가 후산정체(47.5 vs. 16.0%), 대사성질병(18.6 vs. 3.8%) 및 자궁내막염의 발생(62.7 vs. 28.2%)뿐만 아니라 도태율(32.2 vs. 16.5%)이 단태분만 시에 비해 현저하게 증가되었다(P<0.01). 쌍태분만은 분만으로부터 임신까지의 간격에 유의적 인 영향을 미치지 않았으나, 분만계절이 영향을 미쳤는데, 즉 봄에 분만 시가 겨울에 분만 시에 비해 임신율이 감소되 었으며(AHR = 0.80; P = 0.01), 또한 자궁내막염의 발생이 임신율의 감소를 초래하였다(AHR = 0.46, P<0.01). 로지스 틱 분석은 산차의 증가(P<0.01)와 수태 전 번식호르몬의 사용(OR = 1.84, P<0.05)이 쌍태분만의 위험요인임을 나타 내었다. 결론적으로, 젖소에서 쌍태분만은 산후질병 발생과 도태의 증가에 의한 심한 경제적 손실을 초래하므로, 쌍태 분만에 대한 적절한 관리 및 고산차 소 및 번식호르몬 사용에 대한 적절한 통제를 통한 위험요인을 감소시키는 것이 요구된다.

주요어 : 쌍태분만, 산후질병, 도태, 젖소