

# Factors affecting stillbirth and effects of stillbirth on subsequent lactation performance in a Holstein dairy herd in Isfahan

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## Summary

The purposes of this study were to estimate the effects of risk factors on stillbirth parturition and evaluate the effects of stillbirth on lactation performance in Holstein dairy cows. Data were obtained from a dairy cattle herd located in Isfahan province, Iran. The logit of the probability of stillbirth was modeled using logistic regression and the effect of stillbirth on lactation performance was analyzed through mixed linear model using 12283 lactation records on 5201 cows. The results showed that 6% of the calves born die within 48 h of birth. The results of logistic regression analysis demonstrated that the frequency of stillbirth is significantly influenced ( $P < 0.05$ ) by calving year, parity number and calving difficulty. The stillbirth frequency was found to be significantly higher for first parity cows, ( $P < 0.05$ ). Also, calving difficulty was shown to be significantly associated with increased risk of stillbirth ( $P < 0.05$ ). Mixed linear model analysis results demonstrated that stillbirth significantly reduced milk and fat yield ( $P < 0.05$ ), but had no significant impact on milk fat percentage ( $P \geq 0.05$ ). The 305-d milk and fat yields for cows with stillbirth were estimated to be  $8274.17 \pm 100.79$  and  $245.60 \pm 3.7$  kg compared to  $8865.60 \pm 88.16$  and  $259.36 \pm 3.2$  kg for cows that had a live calf, respectively.

**Key words:** Stillbirth parturition, Calving difficulty, Logistic regression, Risk factor, Holstein dairy cows

## Introduction

Stillbirth parturition is defined as calves that are either born dead, and or die within 48 h after calving (Berry *et al.*, 2007; Bicalho *et al.*, 2007; Gundelach *et al.*, 2009). The incidence of stillbirth parturition in dairy cows seems to have increased in recent years (Meyer *et al.*, 2000, 2001; Hansen *et al.*, 2004; Bicalho *et al.*, 2007). During the past 20 years an increase from about 6 to 10.3% has occurred in the incidence of stillbirth in the U.S (Berglund *et al.*, 2003). Meyer *et al.* (2001) have reported that, from 1985 to 1996 the incidence of stillbirth increased from 9.5 to 13.2% in primiparous and 5.0 to 6.6% in multiparous cows. The overall incidence of calf stillbirth in Holstein cows of Iran was reported to be 4.9% and varied among herds from 2.9 to 9.8% (Ghavi Hossein-Zadeh *et al.*, 2008).

Stillbirth parturition does constitute considerable financial losses to the dairy farmer in different ways. This disorder is associated with increased risk of developing metritis and retained placenta and has a considerable negative effect on lactation performance, conception rate and longevity. Stillbirth parturition reduces the number of calves for sale and replacement (Correa *et al.*, 1993; Emanuelson *et al.*, 1993; Maizon *et al.*, 2004).

Economic losses of stillbirth to the dairy industry in the U.S. just due to the loss of replacement heifers were estimated to be \$125 million per year (Meyer *et al.*, 2001). It was reported that cows with stillbirth parturition were at a 41% increased hazard to die or to be culled from the herd than cows without stillbirth (Bicalho *et al.*, 2007). Also, earlier studies showed that calving associated with dead calves leads to lower lactation performance when compared with

live calving (Berry *et al.*, 2007; Bicalho *et al.*, 2007). One or more factors (e.g., parity, age at first calving, calving season and twinning) may contribute to a complex contribution of events when the final outcome is a stillborn calf.

Identification of the risk factors associated with stillbirth can aid in optimizing herd reproductive efficiency. Also, the estimation of the effects of the disease on lactation performance, fertility and survival has great importance to assess the cost-benefits of diagnosis, treatments and prevention efforts.

To the authors' knowledge, there is no scientific report on the impact of stillbirth parturition on lactation performance in Iran. The objectives of the present study were to estimate the incidence of stillbirth parturition frequency, evaluate the effect of some risk factors affecting stillbirth incidence and predict effects of stillbirth on subsequent lactation performance in a Holstein dairy herd in Isfahan.

## Materials and Methods

### Data and definition of traits

Data were obtained from a dairy cattle herd with reliable records on Holstein dairy cows born between 1996 and 2003. The herd is located in Isfahan province, Isfahan, Iran. The herd used in this study is big purebred Holsteins, and consists of 6000 male and female calves, heifers and cows. The herd is managed under conditions similar to most other developed countries and is under official performance and pedigree recording. Cows are milked 3 times a day. The main components of the dairy ration consist of corn silage, alfalfa, barley grain, fat powder, beet pulp, and feed additives, and cows were fed by total mixed ration.

Information on calf, sire and dam identification, as well as occurrence of stillbirth, calving date, parity, age at the first calving, twinning, calving difficulty, 305-d milk yield, 305-d fat yield and fat percentage were extracted from the records kept at the herd's database.

Initially, 16520 lactation records on 6622 cows were available, of which 12283 on 5201 cows, had information on calving

events (calving date, twinning and parity) and calf condition (stillbirth or live birth and calving difficulty).

Stillbirth was defined in two categories; survived 48 h after birth and dead at birth or within 48 h after birth. Calving ease scores (CES) consisted of 5 ordered categories:

CES 1 = Easy calving without assistance,

CES 2 = Easy calving with assistance,

CES 3 = Difficult calving (mechanical assistance),

CES 4 = difficult calving (veterinary assistance),

CES 5 = Calving requiring caesarean (Simianer *et al.*, 2001).

In the presented study, there were a total of 6 cows that had a CES of 5, and these cows were collapsed into the CES 4 group.

## Methodology

Using multivariable logistical regression procedure, the potential effects of environmental risk factors such as calving year, calving season, parity, age at the first calving, calving ease scores twinning and their interactions on stillbirth rate were examined, simultaneously. The multivariable logistical regression model was constructed using the GENMOD procedure of SAS (1999).

Data on parity number of cows was grouped in four classes (1, 2, 3 and  $\geq 4$ ) and data on calving year was grouped in six classes ( $\leq 2002$ , 2003-2006 and  $\geq 2007$ ). Significance of main effects and interaction terms were declared at  $P < 0.05$  based on the Wald Chi-square test.

The potential effects of stillbirth parturition, twin calving, parity, calving year and calving season, age at the first calving and calving difficulty on subsequent lactation performance (305-d milk and fat yield as well fat percentage) were analyzed through mixed linear models using the MIXED procedure of SAS (1999). In the applied multivariate mixed linear models and logistic regression, animal genetic effect was considered as a random effect, while calving year, calving season, age at first calving, twinning, calving ease score, stillbirth parturition, and interaction terms were fitted as fixed effects.

The followed general mixed linear model was used to analyze the effects of environmental factors on the lactation

performance traits.

$$y_{ijklmno} = \mu + (YS)_{ij} + Parity_k + Stillbirth_l + CES_m + b_1(Age)_{ijklmn} + b_2(DIM)_{ijklmno} + Animal_{ijklmn} + e_{ijklmno}$$

Where

$y_{ijklmno}$ : Dependent variable (lactation performance traits),

$\mu$ : Overall mean,

$YS_{ij}$ : Interaction effect of  $i^{th}$  year and  $j^{th}$  season of calving,

$Parity_k$ : Effect of  $k^{th}$  parity,

$Stillbirth_l$ : Effect of  $l^{th}$  kinds of birth (0 = live birth and 1 = stillbirth),

$CES_m$ : Effect of  $m^{th}$  CES,  $b_1$  and  $b_2$ : linear regression coefficients,

Age: Age at the first calving (days),

DIM: Days in milk,

Animal: The random effect of  $n^{th}$  animal belong to  $m^{th}$  CES,  $n^{th}$  Stillbirth,  $k^{th}$  parity,  $j^{th}$  season of calving and  $i^{th}$  calving year,

$e_{ijklmno}$ : Random residual with an expected value of 0 and normal distribution.

## Results

### Stillbirth frequency and risk factors affecting stillbirth

In total, 12283 calvings were used in the final analysis, of which 94% (11550) of those included live calves and 6% (733) were calvings associated with stillbirths.

The results of logistic regression analysis demonstrated that the frequency of stillbirth is significantly influenced ( $P < 0.05$ ) by calving year, parity number and calving difficulty (Table 1). Calving season, the interaction of calving year and calving season, age at the first calving and twinning showed no significant impact ( $P \geq 0.05$ ) on the incidence of stillbirth.

Significant difference ( $P < 0.05$ ) in stillbirth rate was found between first parity than  $\geq$ second parity cows, ORs (95% CI) = 0.65 (0.54-0.79), 0.57 (0.45-0.74) and 0.75

**Table 1: Estimated odds ratios (95% CI) for the effects of calving year, calving ease score (CES), parity number, calving season, twinning and age at the first calving on reported stillbirth rates of Holstein cows (n = 12283)**

Variables	No calving	%Stillbirth	Odd ratio (95% CI)	P-value
Calving year				
$\leq 2002$	1670	7.60	Referent	
2003	1416	6.78	0.99 (0.75-1.30)	0.95
2004	1614	4.96	0.72 (0.53-0.96)	0.03
2005	1911	5.81	0.88 (0.62-1.26)	0.51
2006	2082	5.19	0.71 (0.50-1.01)	0.06
$\geq 2007$	3590	5.88	0.99 (0.71-1.31)	0.95
CES <sup>1</sup> group				
CES = 1	6270	3.96	Referent	
CES = 2	5412	5.69	1.33 (0.99-1.78)	0.06
CES = 3	554	25.99	7.85 (6.14-10.03)	0.001
CES = 4	47	70.21	53.17 (27.54-102.67)	0.001
Parity number				
Parity = 1	5205	7.97	Referent	
Parity = 2	3493	4.61	0.65 (0.54-0.79)	0.001
Parity = 3	2126	4.00	0.57 (0.45-0.74)	0.001
Parity $\geq 4$	1459	4.93	0.75 (0.56-0.98)	0.04
Calving season				
Spring	2432	5.30	Referent	
Summer	3594	5.82	1.20 (0.95-1.52)	0.12
Autumn	3253	6.03	1.15 (0.91-1.47)	0.23
Winter	3004	6.62	1.17 (0.93-1.48)	0.19
Twinning				
Single	11956	5.98	Referent	
Twin	327	5.50	0.98 (0.60-1.60)	0.97
Age			0.99 (0.99-1.01)	

<sup>1</sup>: Calving ease scores (CES) consisted of 4 ordered categories: CES 1 = easy calving with no help, CES 2 = easy calving with assistance, CES 3 = difficult calving but without veterinary assistance and CES 4 = difficult calving with veterinary assistance

(0.56-0.98) for parity 2 vs. 1, parity 3 vs. 1 and parity  $\geq 4$  vs. 1, respectively (Table 1). The frequency of stillbirth for first, second, third and  $\geq 4$ th parity were estimated to be 7.97% (of 5205), 4.61% (of 3493), 4.00% (of 2126) and 4.93% (of 1459), respectively (Table 1).

Calving difficulty followed parity as the next most important factor causing stillbirth parturition (Table 1). There was no significant difference ( $P \geq 0.05$ ) between the stillbirth rate of cows of CES 1 and CES 2 groups, OR (95% CI) = 1.33 (0.99-1.78), (Table 1). Cows of CES 3 and 4 were found to have significantly higher stillbirth incidence rate than cows of CES 1, ORs (95% CI) = 7.85 (6.14-10.03) and 53.17 (27.54-102.67) for CES 3 vs. 1 and CES 4 vs. 1, respectively, (Table 1). The frequency of stillbirth for CES 1, 2, 3 and 4 were estimated to be 3.96% (of 6270), 5.69% (of 5412), 25.99% (of 554) and 70.21% (of 47), respectively (Table 1).

Cows which calved at year of  $\leq 2002$  were shown to have the highest stillbirth parturition rate (Table 1). The results of statistical analysis showed that the interaction effects have no significant impact ( $P \geq 0.05$ ) on stillbirth frequency.

Of the 12283 births, 11956 and 327 cases (97.44 and 2.66%) were shown to be single and twin, respectively (Table 1).

There was no significant difference ( $P \geq 0.05$ ) between stillbirth incidence single and twin birth, [OR (95% CI) = 0.98 (0.60-1.60)].

### Effects of stillbirth on subsequent lactation performance

The results of mixed linear model analysis of environmental factors effects on lactation performance showed that all examined factors except for CES have significant impacts ( $P < 0.05$ ) on 305-d milk and fat yield (Table 2). Fat percentage was shown to be affected significantly ( $P < 0.05$ ) by the interaction effect of calving year and calving season, but other examined factors had no significant impact ( $P \geq 0.05$ ) on fat percentage (Table 2).

The least square means results showed that, stillbirth parturition significantly ( $P < 0.05$ ) reduced the 305-d milk and fat yield but had no significant effect ( $P \geq 0.05$ ) on milk fat percentage (Table 2). The 305-d milk production for cows that had a stillborn calf was  $8274.16 \pm 100.72$  and  $8865.60 \pm 88.16$  kg for the cows that had a live calf. Therefore, a case of stillbirth parturition reduces 305-d milk yield by  $591.43 \pm 69.19$  kg per cow per lactation (Table 2). The 305-d fat production for cows that had a stillborn calf was  $245.60 \pm 3.7$  kg compared to  $259.36 \pm 3.2$  kg for the cows with live birth. Therefore, a case of stillbirth reduces 305-d

**Table 2: Estimated LSMEANS  $\pm$  SED of the effect of parity, stillbirth, twinning and CES on lactation performance**

Parity number	305-d milk	305-d fat	Milk fat percentage
	Estimated mean $\pm$ SE	Estimated mean $\pm$ SE	Estimated mean $\pm$ SE
<b>Parity</b>			
Parity = 1	8393.58 <sup>c</sup> $\pm$ 96.50	248.51 <sup>c</sup> $\pm$ 3.56	2.97 <sup>ab</sup> $\pm$ 0.03
Parity = 2	8666.71 <sup>a</sup> $\pm$ 91.20	258.26 <sup>a</sup> $\pm$ 3.37	2.98 <sup>a</sup> $\pm$ 0.03
Parity = 3	8666.93 <sup>a</sup> $\pm$ 94.02	254.32 <sup>b</sup> $\pm$ 3.47	2.95 <sup>b</sup> $\pm$ 0.02
Parity $\geq 4$	8522.33 <sup>b</sup> $\pm$ 107.78	248.83 <sup>c</sup> $\pm$ 3.98	2.94 <sup>b</sup> $\pm$ 0.03
<b>Stillbirth</b>			
Yes	8274.17 <sup>b</sup> $\pm$ 100.72	245.60 <sup>b</sup> $\pm$ 3.7	2.98 <sup>a</sup> $\pm$ 0.03
No	8865.60 <sup>a</sup> $\pm$ 88.16	259.36 <sup>a</sup> $\pm$ 3.2	2.94 <sup>a</sup> $\pm$ 0.02
<b>Twinning</b>			
No	8650.61 <sup>a</sup> $\pm$ 82.46	255.80 <sup>a</sup> $\pm$ 3.03	2.97 <sup>a</sup> $\pm$ 0.02
Yes	8489.17 <sup>b</sup> $\pm$ 108.09	249.16 <sup>b</sup> $\pm$ 3.98	2.95 <sup>a</sup> $\pm$ 0.03
<b>CES</b>			
CES 1	8608.65 (70.15) <sup>a</sup>	248.9 (2.48) <sup>a</sup>	2.91 (0.02) <sup>a</sup>
CES 2	8618.7 (64.56) <sup>a</sup>	252.9 (2.3) <sup>a</sup>	2.95 (0.02) <sup>a</sup>
CES 3	8381.9 (92.19) <sup>a</sup>	244.5 (3.26) <sup>a</sup>	2.94 (0.02) <sup>a</sup>
CES 4	8740.9 (329.1) <sup>a</sup>	266.44 (11.5) <sup>a</sup>	3.06 (0.09) <sup>a</sup>

<sup>1</sup>: Calving ease scores. <sup>a, b, c</sup>: Means of each factor within a column, not followed by the same subscript differ ( $P < 0.05$ )

fat yield by  $13.77 \pm 2.57$  kg per cow per lactation (Table 2).

The effect of parity on lactation performance was shown to be significant and least square means results showed that cows in the second parity have the highest 305-d milk yield, 305-d fat yield and fat percentage (Table 2).

Twin calving was shown to have a negative depressive effect ( $P < 0.05$ ) on subsequent 305-d milk and fat yield (Table 2). Table 2 highlights the difference in 305-d milk yield, 305-d fat yield and fat percentage of Holstein dairy cows following twin and single calving. A twin birth is followed by a reduction in 305-d milk and fat yield of  $-161.44 \pm 76.79$  and  $-6.65 \pm 2.85$  kg in the lactation initiated by this birth (Table 2). The regression coefficients of lactation performance regressed on days in milk were shown to be significant ( $P < 0.05$ ). The effect of age at the first calving on 305-d milk was significant, but fat yield and fat percentage were shown to be unaffected by age at the first calving.

## Discussion

The stillbirth parturition rate was estimated to be 6% and shown to be a more important problem in the first lactation calving compared to  $\geq$ second calving. Stillbirth frequency in the second and later lactations drops to half or less than that for the first lactation. This is in accordance with reports of other reports (Meyer *et al.*, 2001; Berglund *et al.*, 2003; Eriksson *et al.*, 2004; Hansen *et al.*, 2004; Berry *et al.*, 2007; Bicalho *et al.*, 2007; Ghavi Hossein-Zadeh *et al.*, 2008; Gundelach *et al.*, 2009).

Stillbirth rate is highest for first calving cows, partly because of a disproportion between the size of the calf and the pelvic area, which causes a difficult calving and increases stillbirth parturition incidence (Meyer *et al.*, 2001; Steinbock *et al.*, 2003; Hansen *et al.*, 2004).

Some previous studies reported a significant increase of stillbirth for calves born as a twin (Cady and van Vleck, 1978; Eddy *et al.*, 1991; Mee, 1991; Day *et al.*, 1995; Ghavi Hossein-Zadeh *et al.*, 2008). The reason may be shorter gestation length and greater incidence of calving difficulty

for cows calving twin as the cause of decreased prenatal viability for calves born as twin. The presented study demonstrated no significant effect of twin and single births on the incidence of stillbirth. The same results were also reported by Gundelach *et al.* (2009).

Earlier studies reported calving season as a significant factor affecting the incidence of stillbirth parturition (Meyer *et al.*, 2001; Johanson and Berger, 2003; Steinbock *et al.*, 2003; Silva del Rio *et al.*, 2007; Ghavi Hossein-Zadeh *et al.*, 2008). Silva del Rio *et al.* (2007) reported greater calf mortality occurred during the cold seasons compared with warmer seasons, however, the presented study did not demonstrate such results.

Difficulty of a birth, dystocia, has been implicated as the major cause of stillbirths (Meijering, 1984; Meyer *et al.*, 2001; Berglund *et al.*, 2003; Steinbock *et al.*, 2003; Lombard *et al.*, 2007; Gundelach *et al.*, 2009). The results of this study demonstrated that there is a significant association between calving difficulty and stillbirth incidence. Incompatibility between calf size and dam size, as well as pelvic and vulvar conformation are factors likely to have an important impact on calving difficulty. On the other hand, calving difficulty increases the probability of stillbirth due mainly to trauma and anoxia, a lack of oxygen.

Several studies have implicated stillbirth as contributing factors to reduce subsequent lactation performance (Dematawewa and Berger, 1997; Rajala-Schultz and Grohn, 1998). In this study, 305 days milk and fat yields were estimated to be decreased by  $591.43 \pm 69.19$  and  $13.77 \pm 2.57$  kg per cow per lactation due to stillbirth, respectively. The biology of how stillbirth calving may affect milk production is thus far unknown. As mentioned by Mangurkar *et al.* (1984), it is possible that stillbirth initiates a cascade of effects that will detrimentally affect the cow's performance, but it is also possible that there are common causes to calf mortality and dams' poor performance. For instance, in humans maternal obesity is a well-known risk factor for stillbirth. Obese pregnant women are at 2.1 times higher odds of having stillbirths when compared to

normal weight pregnant woman (Chu *et al.*, 2008).

A twin birth is followed by a reduction in 305-d milk and fat yield of  $-161.44 \pm 76.79$  and  $-6.65 \pm 2.85$  kg in the lactation initiated by this birth, which are in accordance with that obtained by Meyer *et al.* (2000). The negative genetic correlation between reproduction and production traits can explain some part of this result.

The present study demonstrated that primiparous cows had significantly higher stillbirth rates compared with multiparous cows. Following parity, dystocia was the next most influential predictor of whether a calf would be alive or stillborn, therefore, any management intervention to reduce the incidence of difficult births should reduce the incidence of stillbirth.

One of the best management practices to reduce stillbirth parturition may be utilizing sire and daughter calving ease information when selecting sires to breed heifers. Herd managers should review calving procedures with their veterinarian to assure that proper timing and calving assistance techniques are used when providing assistance during parturition. In addition, providing a good environment for heifers and cows to minimize stress before parturition can reduce stillbirth incidence.

It was also concluded that stillbirth significantly decreased milk and fat yields and these effects (loss of  $591.43 \pm 69.19$  kg milk and  $13.77 \pm 2.57$  kg fat per cow per lactation) are comparable with other diseases with recognized impact (such as mastitis and lameness). Therefore, the losses from stillbirths are far greater than just the value of the stillborn calf and in any economical evaluation of stillbirth, not only the lost calf, the reduced survival, and the increased days open should be taken into account, but also the decreased milk and fat yield.

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