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Non-Genetic Factors Affecting Stillbirth and its Effects on Longevity, Production, and Reproductive Performance in Holstein Cows of Iran

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ABSTRACT-The aim of this study was to determine the factors affecting stillbirth and the consequences of stillbirth on longevity, production, and reproductive performance in Holstein cows of Iran. Calving records from March 2000 to April 2009 comprising of 220,043 calvings on 102,199 cows in 33 herds were used. The results showed that 4.06% of the newborn calves died within 48 h of birth. The stillbirth rate was significantly higher for first parity cows (P<0.01). Calving difficulty significantly increased the risk of stillbirths (P<0.01). The frequency of stillbirth was lower for female calves than for males and was higher for twins than for singletons (P<0.01). Stillbirth significantly (P<0.01) reduced 305 d milk, fat and protein yield by 222 (±31), 8 (±1.12), and 6 (±1.01) kg per cow per lactation, respectively. Milk protein percentage, but not fat percentage, was affected by stillbirth (P<0.01). The mean calving interval for cows experiencing stillbirth was significantly longer than for those with live calves [410.3 (±1.17) *vs*. 405.5 (±1.05) d (P<0.01), respectively]. The overall mean longevity was affected by stillbirth while the shortest lifetime was observed for cows with stillbirth at their first parity (P<0.01).

Keywords: Calving Interval, Holstein Cows, Lactation Performance, Stillbirth

INTRODUCTION

Stillbirth, the death of a calf prior to, during, or within 24 to 48 h of parturition, is a major concern in dairy cow management (21). Increased frequency of stillbirth has been reported in several countries during the last decades (2, 4, 12 and 18). Stillbirth negatively affects the dairy industry through increased risk of developing metritis, and retained placenta, longer rebreeding intervals, increased hazard of involuntary culling, reduced number of calves for sale, fewer replacement heifers (3, 6, 8, 14, 16 and 19), and lower milk production (2 and 4).

Holstein Friesian is the dominant dairy cattle breed in Iran, but very few comprehensive reproductive studies have been carried out concerning this population (25). The mean stillbirth rate in 40 dairy farms in the northwest of Iran was 3.7% (20). In the same study, the twinning rate, calving difficulty, and placental retention were 4.0%, 4.8%, and 9.2%, respectively. Ghavi Hossein-Zadeh et al. (9) using the

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calving records of 16 dairy farms over an 8-year period (supplied by the Animal Breeding Center of Iran) calculated stillbirth and abortion rate, twinning rate and sex ratio.

The present study aimed at determining the non-genetic factors affecting stillbirth and the effects of stillbirth on longevity, lactation performance, and reproductive performance of Holstein cows in Iran.

MATERIALS AND METHODS

Data

Calving records from March 2000 to April 2009 comprising of 220,043 calving events of 102,199 cows in 33 dairy herds, supplied by the Animal Breeding Center of Iran, were used. The herds were purebred Holsteins, managed under conditions similar to most developed countries and under official performance and pedigree recording. Cows were fed a total mixed ration, with the main components of the dairy ration being corn silage, alfalfa, cotton seed, cotton seed meal, barley grain, canola meal, wheat bran, fat powder, beet pulp, and feed additives.

Traits Definition

Stillbirth was coded “D0” when a calf was born alive as a singleton or when both calves were born alive in twin births; otherwise, stillbirth was coded as “D1”. The combination of calf sex and birth number (single *vs*. twin) was shown by SST and coded as SM for male and SF for female in singleton births, TMM for male pairs, TFF for female pairs and TMF for mixed-sex pairs in twin births. Calving ease (CES) was coded CES1= no problem, CES2= slight problem, CES3= needed assistance, CES4= considerable force, and CES5= extreme difficulty with surgery. Longevity was defined as the number of days from birth to cull (disposal age), number of days from first calving to death (productive life), and lactation number at culling (disposal parity). Data on cow parity were grouped into five classes; namely parity 1, 2, 3, 4, and ≥5. The combinations of stillbirth and parity of first stillbirth were coded as NS for cow with no stillbirth, and LNS1, LNS2, LNS3, LNS4, and LNS ≥ 5 for cows with their first stillbirth at 1st, 2nd, 3rd, 4th, and ≥ 5th parity.

Statistical Methods

The effect of risk factors on the incidence of stillbirth was analyzed using a multivariate logistical regression model in which the animal effect was considered as random while the effects of herd, calving year, calving season, parity, CES groups, SST groups, and age at the 1^{st} calving were considered as fixed. The model was constructed using the maximum likelihood method through the GENMOD procedure (22). Reference categories for the comparison of odds ratios (ORs) for each effect were spring, parity 1, CES1 and SM.

The potential consequential effects of stillbirth on production and reproductive performance were analyzed through mixed linear models using the MIXED procedure (22). In the models, the sire effect was considered as random while the interaction effect of herd, year and season of calving, parity, CES groups, SST groups, stillbirth and age at the 1^{st} calving, were considered as fixed effects.

The potential consequential effect of stillbirth on lifetime was considered through mixed linear models using the MIXED procedure (22). These cows had been disposed at the time of the analyses. In the models, the sire effect was considered as random while the interaction effect of herd, year and season of cull, interaction of the stillbirth and parity of first stillbirth, and the age of the 1st calving were considered as the fixed effects.

RESULTS

Stillbirth Frequency and Risk Factors Affecting Stillbirth

Of the 220,043 calvings, 4.06% were associated with stillborn calves, while the incidence of stillbirth by herd ranged from 0.33% to 9.97%. The estimated odds ratios (95% CI) for factors affecting stillbirth are presented in Table 1. The incidence of stillbirth was significantly influenced by the parity (P<0.01), being 6.09%, 2.96%, 3%, 3.04%, and 3.31%, for the first, second, third, fourth and ≥ fifth parity cows.

Table 1. Estimated odds ratios (95% CI) for the effects of combination of calf sex and birth number, calving difficulty, parity and calving season on reported calf stillbirth

Variable	Number of calving	Odds ratio (95% CI)	*P*-value
SST[1]			<0.01
SM[2]	108038	Reference	
SF[3]	105363	0.79 (0.74-0.83)	<0.01
TMM[4]	1968	1.87 (1.49-2.32)	<0.01
TMF[5]	3173	1.59 (1.32-1.92)	<0.01
TFF[6]	1501	1.47 (1.11-1.94)	<0.01
CES[7]			<0.01
CES1	183728	Reference	
CES2	12945	86.65 (74-96)	<0.01
CES3	20023	177.9 (160-196)	<0.01
CES4	3120	401.4 (347-462)	<0.01
CES5	227	320.0 (213-478)	<0.01
Parity			<0.01
Ln=1	72593	Reference	
Ln=2	54843	0.83 (0.78-0.89)	<0.01
Ln=3	38033	0.89 (0.82-0.96)	<0.01
Ln=4	24590	0.88 (0.79-0.96)	<0.01
Ln≥5	29984	0.91 (0.82-0.98)	<0.05
Calving season			<0.05
Spring	51019	Reference	
Summer	62531	1.12 (1.03-1.21)	<0.05
Fall	56358	1.07 (0.98-1.15)	≥0.05
Winter	50135	1.03 (0.95-1.11)	≥0.05

1. **The combination of calf sex and birth number**
2. **SM= male in singleton births**
3. **SF = female in singleton births**
4. **TMM = male pairs in twin births**
5. **TMF = mixed-sex pairs in twin births**
6. **TFF = female pairs in twin births**
7. **Calving ease (CES) was coded as; CES1= no problem, CES2= slight problem, CES3= needed assistance, CES4= considerable force, CES5= extreme difficulty with surgery.**

Calving ease score was the next most important factor affecting stillbirth ($P<0.01$). The frequency of stillbirth for CES1, CES2, CES3, CES4, and CES5 was 0.39%, 15.58%, 24.32%, 39.29%, and 39.21%, respectively. The highest stillbirth frequency was observed when parturition occurred during the summer months [ORs (95% CI) = 1.12 (1.03-1.21), 1.07 (0.98-1.15), and 1.03 (0.95-1.11) for calving season of summer, fall, and winter *vs.* spring, respectively].

The overall twinning rate was 3.02%, and the percentage of singleton male births was 50.63%. Of the 6,642 twin births, 29.63%, 22.60%, and 47.77% were male, female and mixed-sex pairs, respectively. The incidence of stillbirth for the combination of calf sex and birth number was 5.14%, 2.74%, 8.28%, 7.12%, and 5.93% for SM, SF, TMM, TMF, and TFF, respectively. Based on the data in singleton pregnancies, female calves tended to be at a lower risk of being stillborn than male calves [OR = 0.79 (0.74-0.83) for SF *vs*. SM]. The stillbirth risk was also considerably higher for twin births [OR = 1.87 (1.49-2.32), 1.59 (1.32-1.92) and 1.47 (1.11-1.94) for TMM, TMF and TFF *vs*. SM, respectively]. The frequency of stillbirth differed according to the calving year but no consistent phenotypic trend was observed for stillbirths from 2000 to 2009 (Fig 1).

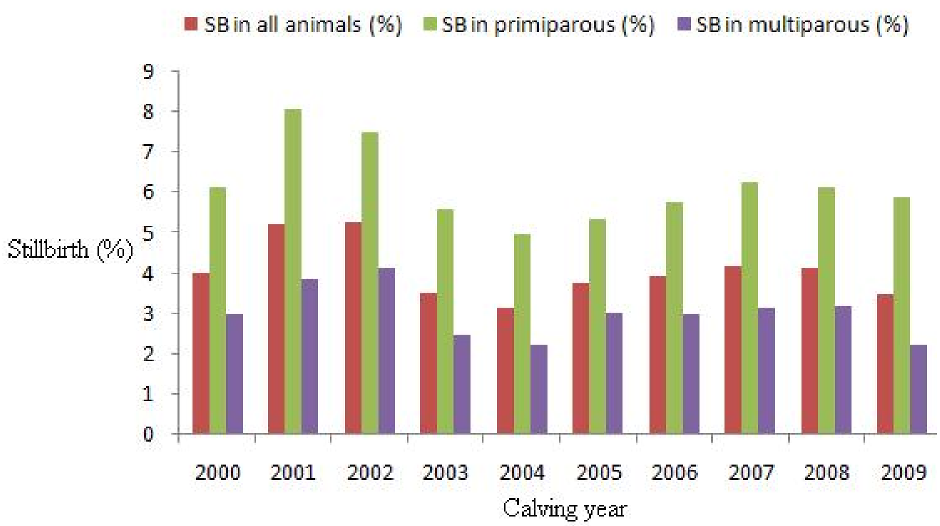

Fig. 1. The frequency of stillbirths (SB) in primiparous, multiparous, and all cows according to the calving year

Effects of Stillbirth on Lactation and Reproductive Performance

The potential consequential effects of stillbirth on lactation and reproductive performance are presented in Table 2. The 305-d milk, fat, and protein yields were significantly reduced by stillbirth ($P<0.01$). The least squares means (±SE) of 305-d milk, fat, and protein yield were 7329 (±56), 240 (±2), and 232 (±1.8) kg for cows that had a stillborn calf while the corresponding values for cows with live calves were 7551 (±51), 248 (±1.8), and 238 (±1.7) kg, respectively (Table 2). The overall means (±SD) of milk-fat and protein percentage were 3.33% (±0.58), and 3.06% (±0.34), respectively. Stillbirth significantly influenced milk-protein percentage ($P<0.01$; Table 2). The least squares means (±SE) of calving interval for cows with a stillborn calf and for those with a live calf were 410.3 (±1.17), and 405.5 (±1.05) d, respectively ($P<0.01$).

Effects of Stillbirth on Cow Longevity

The average (±SD) disposal age, productive life and disposal parity, were 1987 (±825) d, 1215 (±820) d, and 2.90 (±1.46) lactations per cow, respectively (Table 3). The results showed that 9.2% of all culled cows had experienced at least one stillbirth event during their life. The least squares means of longevity for cows which had experienced stillbirth at the first calving were shorter compared to cows which had experienced stillbirth at other parities or cows which had not experienced stillbirth at any parity (P<0.01).

Table 2. Estimated LSMEANS and standard errors of the effect of parity, combination of calf sex and birth number, calving difficulty and stillbirth parturition on production and reproductive performance

Variables	Milk(Kg)	Fat(Kg)	Fat %	Protein(Kg)	Protein %	CI(day)
Parity	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**	**P<0.01**
Ln=1	7476(51)^{b}	243(1.8)^{c}	3.28(.01)^{d}	235(1.7)^{b}	3.10(.01)^{c}	406.5(1.1)^{c}
Ln=2	7641(52)^{a}	249(1.8)^{a}	3.30(.01)^{c}	241(1.7)^{a}	3.12(.01)^{a}	408(1.1)^{b}
Ln=3	7616(52)^{a}	250(1.9)^{a}	3.32(.01)^{b}	240(1.7)^{a}	3.12(.01)^{a}	407.7(1.1)^{b}
Ln=4	7434(54)^{c}	244(1.9)^{b}	3.33(.01)^{b}	234(1.8)^{c}	3.11(.01)^{b}	407.5(1.1)^{b}
Ln≥5	7032(54)^{c}	234(1.9)^{d}	3.37(.01)^{a}	223(1.8)^{d}	3.13(.01)^{a}	410(1.1)^{a}
SST[1]	**P≥0.05**	**P≥0.05**	**P≥0.05**	**P≥0.05**	**P≥0.05**	**P<0.01**
SM[2]	7440(48)^{a}	244(1.7)^{a}	3.33(.01)^{a}	235(1.6)^{a}	3.11(.01)^{a}	406(.94)^{b}
SF[3]	7434(48)^{a}	245(1.8)^{a}	3.33(.01)^{a}	234(1.6)^{a}	3.11(.01)^{a}	405(.94)^{c}
TMM[4]	7424(74)^{a}	243(2.6)^{a}	3.31(.02)^{a}	234(2.4)^{a}	3.10(.02)^{a}	409(1.7)^{a}
TMF[5]	7417(65)^{a}	242(2.3)^{a}	3.32(.02)^{a}	235(2.1)^{a}	3.13(.02)^{a}	408(1.4)^{a}
TFF[6]	7483(79)^{a}	245(2.8)^{a}	3.22(.02)^{a}	235(2.6)^{a}	3.11(.02)^{a}	411(1.8)^{a}
CES[7]	**P<0.01**	**P≥0.05**	**P≥0.05**	**P≥0.05**	**P≥0.05**	**P<0.01**
CES1	7567(33)^{a}	247(1.1)^{a}	3.32(.01)^{a}	237(1.0)^{a}	3.10(.01)^{a}	399(.65)^{c}
CES2	7500(37)^{b}	245(1.3)^{a}	3.32(.01)^{a}	236(1.2)^{a}	3.11(.01)^{a}	402(.79)^{b}
CES3	7526(34)^{b}	246(1.2)^{a}	3.32(.01)^{a}	236(1.1)^{a}	3.11(.01)^{a}	412(.69)^{a}
CES4	7386(52)^{c}	246(1.8)^{a}	3.34(.01)^{a}	235(1.6)^{a}	3.11(.01)^{a}	409(1.2)^{a}
CES5	7220(206)^{abc}	234(7.4)^{a}	3.30(.06)^{a}	229(6.9)^{a}	3.14(.04)^{a}	417(4.3)^{a}
Stillbirth	**P<0.01**	**P<0.01**	**P≥0.05**	**P<0.01**	**P<0.01**	**P<0.01**
D0 (No)	7551(51)^{a}	248(1.8)^{a}	3.30(.02)^{a}	238(1.7)^{a}	3.10(.01)^{b}	405.5(1.05)^{b}
D1(Yes)	7329(56)^{b}	240(2.0)^{b}	3.33(.02)^{a}	232(1.8)^{b}	3.12(.01)^{a}	410.3(1.17)^{a}

1. **The combination of calf sex and birth number**
2. **SF=female in singleton births**
3. **SM =male in singleton births**
4. **TFF = female pairs in twin births,**
5. **TMM= male pairs in twin births**
6. **TFM = mixed-sex pairs in twin births.**
7. **Calving ease (CES) was coded as; CES1= no problem, CES2= slight problem, CES3= needed assistance, CES4= considerable force, CES5= extreme difficulty with surgery.**

[a,b,c] **Within each column and for each variable, the least squares means having different superscript differ (P < 0.05)**

RESULTS AND DISCUSSION

Of the 220,043 calvings, 4.06% were stillborn calves, while the incidence of stillbirth by herd ranged from 0.33% to 9.97%. The incidence of stillbirth for primiparous and multiparous cows was 6.09% and 3.05%, respectively. In another study, the incidence of stillbirth in Holstein herds in Iran was 4.9% (9). Meyer et al. (18) and Bicalho et al. (3) showed that about 7% of the U.S. Holstein calves died within 48 h of birth. Meyer et al. (18) reported significant differences in the stillbirth percentage between primiparous (11.0%) and multiparous cows (5.7%).

Table 3. Estimated LSMEANS and standard errors of the effect of the first parity of stillbirth on longevity

Variable	disposal age[1]	productive life [2]	disposal parity [3]
The parity of first stillbirth	**P<0.01**	**P<0.01**	**P<0.01**
“NS”[4]	**1665(30)^{d}**	**893(30)^{d}**	**2.35(.04)^{c}**
” LNS1”[5]	**1489(33)^{f}**	**717(32)^{f}**	**1.90(.05)^{e}**
” LNS1”[6]	**1603(34)^{e}**	**831(34)^{e}**	**2.28(.06)^{d}**
” LNS1”[7]	**1742(35)^{c}**	**970(35)^{c}**	**2.75(.06)^{b}**
” LNS1”[8]	**1856(38)^{b}**	**1085(38)^{b}**	**3.18(.07)^{a}**
” LNS≤5”[9]	**2112(36)^{a}**	**1340(36)^{a}**	**3.16(.06)^{a}**
Overall means±(SD)	**1987(825)**	**1215(820)**	**2.90(1.46)**

a,b,c within each column, the least squares means having different superscript differ ($P < 0.05$)

Stillbirth incidence in the second and later parities was about 50% lower than for the first parity. This finding is in line with the data reported by others (2, 3, 12 and 18). Higher stillbirth in the first parity may be partly due to the disproportion between the calf size and the pelvic area (3). Calving ease score followed parity as the next most important factor affecting stillbirth incidence. Dystocia has been considered as the major cause of stillbirths (2, 3, 14, 17, and 19). We found that CES1 and CES2 represented 15.97% of all stillbirth calvings as compared to the 67.9% reported by Bicalho et al. (3). Johanson and Berger (13) concluded that 49% of the perinatal mortality was associated with unassisted births. According to Berry et al. (2), there was an 8-time greater likelihood of stillbirth when assistance at calving was required. Incompatibility between calf size and dam size, as well as pelvic and vulvar conformation are factors likely to have great impact on calving difficulty, which increases the incidence of stillbirths, mainly due to trauma and anoxia.

The largest stillbirth frequency was observed when parturition occurred during the summer months. Silva del Rio et al. (23) reported greater calf mortality in the U.S. Holstein cows during the colder months. The discrepancies between studies may reflect true biological differences across various populations, differences among the applied statistical methods, and differences among the studied sample size.

Twinning has often been associated with more frequent stillbirth, and it has been reported that bilateral twin pregnancies result in lower incidence of stillbirth and fetal malpresentation than unilateral twin pregnancies (10). Due to their larger size at birth, male calves are more prone to conditions that result in stillbirth. The risk was also considerably higher for twin births. Silva del Rio et al. (23) found that the overall singleton mortality was not different between genders (7.2%, and 7.3% for male and female calves, respectively). Gundelach et al. (11) reported that calf gender was not associated with stillbirth and that there was no difference in stillbirth rates between twin and single calves. Berry et al. (2) reported that the odds of at least one stillbirth were 11.9 times greater in twins than for a singleton. Silva del Rio et al. (23) reported that in 28.2% of the twin birth, one or both calves had died, compared with the 7.2% for singleton births. This may be due to shorter gestation length and greater incidence of dystocia in cows calving twins as the cause of decreased perinatal viability.

Our results showed that the frequency of stillbirth differed according to the calving year but there was no consistent phenotypic trend for stillbirths. In contrast, the incidence of stillbirth in dairy cows has consistently increased in recent decades (1, 3, 7, 12, 19 and 21). Berglund et al. (1) reported that an increase of about 6% to 10.3% has occurred in stillbirth in the U.S. Holstein cows during the past 20 years. Meyer et al. (19) reported that from 1985 to 1996 the incidence of stillbirth increased from 9.5% to 13.2% in primiparous and from 5.0% to 6.6% in multiparous cows in the U.S.A. These findings are also in line with the reports indicating gradual decline in dairy cow fertility during the last decades (15). Cole et al. (7) reported that the mean incidence of stillbirth from 1980 to 2005 was 10.5% in heifers and 5.9% in multiparous cows in the U.S.A. The overall frequency of stillbirth in Danish Holstein cows increased from 7.1% to 9.0% from 1985 to 2002 (12).

The results of the present study demonstrated that following the birth of a stillborn calf, the average 305-d milk, fat, and protein yields were reduced by 222 (±31), 8 (±1.12), and 6 (±1.01) kg per cow per lactation, respectively. Bicalho et al. (4) found that the mean 305-d milk yield for a cow with a stillborn calf was decreased by 323.3 kg per cow per lactation. Mangurkar et al. (17) reported a loss from perinatal deaths of 100–400 kg of milk, 4–11.5 kg of fat, and 2.5–13 kg of protein. Berry et al. (2) demonstrated that cows that had a stillborn calf yielded 51.9 kg less milk, during the first 60 days of lactation. Chassagne et al. (6) reported a significantly reduced average 305-d milk yield in cows with a stillborn calf (5582 kg) compared with the cows with live calves (6140 kg). The mean calving interval for cows that had a stillborn calf was increased by 4.78 (±0.73) d per cow per lactation. Bicalho et al. (3) found that the mean days open was significantly smaller for cows that had live calves compared to those with stillbirths (186 *vs.* 212 days). They reported that the hazard ratio for being diagnosed pregnant was 24.1% lower for cows that had a stillborn calf when compared with those with a live calf (3).

The mean longevity for cows, which had experienced stillbirth at the first calving, was shorter compared to the cows which had experienced stillbirth at other parities, or cows which had not experienced stillbirth at any parity. Bicalho et al. (3) reported that the hazard rate of death/cull was 41% higher for cows with a stillborn calf compared to those with live calves. Hormonal imbalances before parturition have been associated with stillbirth (16 and 17). Recently, Sorge et al. (24) reported that serum estradiol-17β concentration was lower in heifers with stillborn calves. The lower estradiol-17β concentration might indicate abnormalities in the placenta, or hormonal signals from the calf to the placenta before calving (24). Recently, Brickell et al. (5) reported that polymorphisms in the leptin gene in dairy heifers were associated with 2-fold differences in perinatal mortality, which they defined as stillbirths, and mortality within 24 h of parturition.

In conclusion, the present study showed that cows with stillborn calves had longer calving interval and shorter lifetime. Stillbirth also was associated with decreased milk, fat and protein yields. Therefore, the losses from stillbirths are far greater than just the value of the stillborn calf, and for economical evaluation of stillbirth, the value of the lost calf, lower cow longevity, increased days open, and decreased milk, fat and protein yield should be taken into account.

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عوامل غیر ژنتیکی موثر بر مرده‌زایی و اثر مرده‌زایی بر طول عمر، عملکرد تولیدی و تولید مثلی گاوهای هلشتن ایران

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چکیده- در این پژوهش برای بررسی عوامل موثر بر مرده‌زایی و اثر مرده‌زایی بر طول عمر، عملکرد تولیدی و تولید مثلی در گاوهای هلشتن ایران، ۲۲۰۰۴۳ رکورد زایش مربوط به ۱۰۲۱۹۹ گاو از ۳۳ گله گاو که در بین سال‌های ۱۳۷۹ تا ۱۳۸۸ جمع‌آوری شده بودند، استفاده شد. نتایج نشان داد که در ۴/۰۶ درصد از زایش‌ها، گوساله‌ها یا مرده به دنیا آمده بودند و یا تا ۴۸ ساعت پس از تولد مرده بودند. نتایج حاصل از رگرسیون لجستیک نشان داد که نرخ مرده‌زایی در گاوهای شکم نخست در مقایسه با دیگر شکم‌های زایش به طور معنی‌داری بالاتر بود (P<0.01). سخت زایی به طور معنی‌داری نرخ مرده‌زایی را افزایش داده است (P<0.01). احتمال مرده‌زایی در گاوهای دارای گوساله‌ی نر بیشتر از ماده بود و همچنین مرده‌زایی در زایش‌های دوقلو بیشتر از زایش‌های تک‌قلو بود (P<0.01). مرده‌زایی در زایش‌های فصل تابستان بیشتر از دیگر فصل‌ها بود (P<0.01). تولید شیر، چربی و پروتئین در گاوهای مرده‌زا به ترتیب (۳۱±)۲۲۲، (۱/۱۲±)۸، و (۱/۰۱±)۶، کیلوگرم کمتر از گاوهایی بود که گوساله آنها مرده به دنیا آمده بود (P<0.01). مرده‌زایی تاثیر مثبتی بر درصد پروتئین داشت (P<0.01) اما بر درصد چربی تاثیری نداشت (P≥0.05). مرده‌زایی باعث افزایش فاصله گوساله‌زایی و کاهش طول عمر گاو در گله شد (P<0.01).

واژه‌های کلیدی: عملکرد تولیدی، فاصله زایش، گاوهای هلشتن، مرده‌زایی

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