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The effect of maternal inbreeding on incidence of twinning, dystocia and stillbirth in Holstein cows of Iran

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Summary

The purpose of this study was to investigate the effect of maternal inbreeding on incidence of twinning, dystocia and stillbirth in Holstein cows of Iran. Calving records from March 2000 to April 2009 comprising of 365,021 calvings on 153,802 cows from 86 dairy herds were used. The mean level of inbreeding was 0.7%, less than 37% of all animals were inbred, and a small proportion (3.45%) of inbreed animals had inbreeding coefficients greater than 5%, with a maximum inbreeding coefficient of 38%. The overall average twinning and stillbirth rates were 2.7 and 3.78%, respectively. Dam parity and calving season significantly affected the incidence of twinning ($P<0.01$). The twinning rate at parities 1, 2, 3, 4 and ≥5 were 0.72, 3.4, 4.51, 4.70 and 5.23%, respectively ($P<0.01$). The incidence of stillbirth was significantly influenced by parity, calving season, calf sex and twinning ($P<0.05$). Dystocia scores of ≥3 were less common amongst older cows. Gender of the calf was significantly associated with dystocia, whereas the incidence of dystocia was higher in dams delivering male calves ($P<0.01$). First parity cows showed more difficult calving than higher parity cows ($P<0.01$). The results indicated that the degree of maternal inbreeding presently observed in Iranian Holstein herds does not seem to have a significant effect on the twinning, dystocia and stillbirth rates.

Key words: Twinning, Dystocia, Stillbirth, Inbreeding

Introduction

Inbreeding is defined as the probability that 2 alleles at any locus are identical by descent, and occurs when related individuals are mated to each other (Falconer and MacKay, 1996). In recent years, the average inbreeding in Holstein dairy cows seems to be increasing and growing rates of inbreeding are of serious concern to dairy breeders and the industry (Short *et al*., 1992). The reduction in mean phenotypic performance associated with inbred animals is defined as inbreeding depression, and is generally greater in traits associated with fitness and survival (Falconer and MacKay, 1996). Reproductive traits, such as twinning, dystocia and stillbirth, are ethically and economically important, and it is very much in the interest of both farmers and the public that they occur at the lowest possible levels. These traits are components of fitness and often become subject to positive dominance allelic interactions. Stillborn calves, those born dead or dying within 48 h of birth, are of increasing concern to dairy producers. Several studies have shown that twin births were associated with increased dystocia and stillbirth (Berry *et al*., 2007; Silva del Rıo *et al*., 2007). Calving difficulty also affected the profitability in dairy herds (Dematawewa and Berger, 1997).

Estimates of the inbreeding effect on 305-d milk and fat yield in Iranian Holstein cows were reported to be approximately -12 to -13 and -0.39 kg for each 1% inbreeding, respectively (Tohidi *et al*., 2000). Rokouei *et al*. (2010) reported that the effect of inbreeding was significant for all production traits in Iranian Holstein cows. The objective of this study was to investigate the effect of maternal inbreeding on twinning, dystocia and stillbirth in Holstein cows of Iran using the largest official data set available to date.

Materials and Methods

Data, trait definition and individual inbreeding coefficient calculation

The data were collected by dairy producers according to the instructions of the Animal Breeding Center of Iran. The pedigree file, consisting of 1,025,281 animals, was used to calculate the inbreeding coefficient. The data set consisted of records of 365,021 calvings on 153,802 Holstein cows in 86 dairy herds for the period March 2000 to April 2009. The herds were under official performance and pedigree recording.

Farmers, upon observing parturition, subjectively assigned scores according to degree of assistance provided. Recognized dystocia scores were CES1 = no problem, CES2 = slight problem, CES3 = needed assistance, CES4 = considerable force, CES5 = extreme difficulty with cesarean section. When a calf was born alive as a singleton or when both calves were born alive in twin birth, stillbirth was coded as "D0"; otherwise, stillbirth was coded as "D1". The combination of calf sex and birth number (single vs. twin) were shown by SST and coded as SM and SF for male and female sex in singleton births, TMM, TFF and TMF for male pairs, female pairs and mixed-sex pairs in twin births. Data on parity number of cows were grouped into five classes, parities 1, 2, 3, 4 or ≥5. The individual inbreeding coefficient was calculated using the algorithm described by Meuwissen and Luo (1992).

Statistical methods

Linear regressions of inbreeding on traits were tested for significance when inbreeding was treated as a continuous variable. The effect of risk factors and inbreeding on twin birth incidence was analysed using the following multivariable logistical regression model.

Model 1:

$$\text{logit}(y_{ijklmn}) = \mu + \text{parity}_i + \text{herd}_j + \text{year}_k + \text{season}_l + \text{sire}_m + b_1(F)_{ijklmn} + b_2(\text{age})_{ijklmn} + e_{ijklmn}$$

Where, y_{ijklmn} is birth number of cow n in parity i ($parity_i$), with effect of the j^{th} herd ($herd_j$), k^{th} calving year ($year_k$), l^{th} calving season ($sesason_l$), m^{th} service sire (random effect), regression coefficient (b_1) of effect of the inbreeding (F_{ijklmn}), regression coefficient (b_2) of effect of the age at the first calving (age_{ijklmn}), and residual effect of e_{ijklmn}.

The potential effect of inbreeding on stillbirth was analysed using the same multivariable logistical regression model, but the combination effect of calf sex and birth number (SST) was included in the model as categorical effects.

The multivariable logistical regression model was constructed using the maximum likelihood method of the GENMOD procedure (SAS, 2002). Reference categories for the comparison of odds ratios (ORs) for each effect were as follows: spring, parity 1, CES1 and SM.

The potential effect of inbreeding on dystocia was analysed by the following mixed linear models using the MIXED procedure (SAS, 2002).

Model 2:

$$y_{ijklm} = \mu + \text{HYS}_i + \text{parity}_j + \text{sst}_k + (\text{sire})_l + b_1(\text{age})_{ijklm} + b_1(F)_{ijklm} + e_{ijklm}$$

Where, y_{ijklm} is observation of calving difficulty and other components were described previously in model 1.

Results

Inbreeding coefficient

The mean level of inbreeding was 0.7%, and less than 37% of all animals were inbred. A small proportion (3.45%) of inbred animals had inbreeding coefficients greater than 5%, and the maximum inbreeding coefficient was 38%.

Twin birth frequency and its risk factors

The twinning rate was 2.7%, and the percentage of male and female calves among all singleton births was 0.51 and 0.49%, respectively. Of the 9,811 twin births 27.44, 24.02 and 48.54% were of male pairs, female pairs and mixed-sex pairs, respectively. The incidence of twinning across farms ranged from 0.24 to 10.09%.

The highest twinning rate (3.94%) was observed in the summer calvings [ORs (95% CI) = 1.54 (1.36-1.74) for summer vs. spring calving]. The twinning rate in the fall and winter calvings was smaller than in spring [ORs (95% CI) = 0.93 (0.87-0.99)] and 0.84 (0.77-0.91) for fall and winter vs. spring calving, respectively. The percentages of twin births for parities 1, 2, 3, 4 and ≥5 were 0.72, 3.4, 4.51, 4.70 and 5.23%, respectively (Table 1). The twinning rate decreased from 0.75% in 2000 to 0.54% in 2009 for primiparous, and increased from 2.35% in 2000 to 3.79% in 2009 for multiparous cows (P<0.01). Maternal inbreeding and age of the first calving had no impact on twinning (P≥0.05).

Stillbirth frequency and its risk factors

Of 305,057 calvings, 3.78% were stillborn calves, while the incidence of stillbirth by herd ranged from 0.27 to 9.97%. The incidence of stillbirth for primiparous and multiparous cows was 5.40 and 2.65%, respectively.

The estimated odds ratios (95% CI) of factors affecting stillbirth are presented in Table 2. The incidence of stillbirths for the first, second, third, fourth and ≥ fifth parity cows was 5.40, 2.67, 2.59, 2.60 and 2.77%, respectively (P<0.01). Stillbirth incidence in the second and later lactations was lower than that in the first lactation. The largest stillbirth frequency was observed when parturition occurred in winter [ORs (95% CI) = 0.94 (0.88-1.01), 0.99 (0.93-1.06), and 1.08 (1.02-1.16) for the calving seasons of summer, fall, and winter vs. spring, respectively].

The average incidence of stillbirth for singleton and twin birth calving was 3.73 and 5.93, respectively. The incidence of stillbirth for combination of calf sex and birth number was 4.85, 2.57, 7.64, 5.78 and 4.38 for SM, SF, TMM, TMF and TFF, respectively. After single birth, male calves

Table 1: Estimated odds ratios (95% CI) for the effects of parity and calving season on reported calf twin birth

Variable	No. of calving	Odds ratio (95% CI)	P-value
Parity			<0.01
Ln=1	153,668	Referent	
Ln=2	96,646	4.7 (4.18-5.28)	<0.01
Ln=3	58,019	6.22 (5.53-7.0)	<0.01
Ln=4	31,598	6.50 (5.76-7.35)	<0.01
Ln≥5	24,953	6.80 (6.05-7.62)	<0.01
Calving season			<0.01
Spring	83,556	Referent	
Summer	99,289	1.54 (1.36-1.74)	<0.01
Fall	92,221	0.93 (0.87-0.99)	<0.05
Winter	89,818	0.84 (0.77-0.91)	<0.01

Table 2: 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	No. of calving	Stillbirth (%)	Odds ratio (95% CI)	P-value
SST[1]				<0.01
SM[2]	150,250	4.85	Referent	
SF[3]	146,336	2.57	0.51 (0.44-0.58)	<0.01
TMM[4]	2,252	7.64	1.88 (1.55-2.27)	<0.01
TMF[5]	4,166	5.78	1.51 (1.25-1.82)	<0.01
TFF[6]	2,053	4.38	1.12 (0.87-1.47)	≥0.05
Parity				<0.01
Ln=1	126,017	5.40	Referent	
Ln=2	80,987	2.67	0.45 (0.40-0.51)	<0.01
Ln=3	48,925	2.09	0.45 (0.40-0.51)	<0.01
Ln=4	27,236	2.60	0.46 (0.41-0.51)	<0.01
Ln≥5	21,892	2.77	0.51 (0.45-0.58)	<0.01
Calving season				<0.05
Spring	69,844	3.70	Referent	
Summer	82,094	3.51	0.94 (0.88-1.01)	≥0.05
Fall	78,566	3.76	0.99 (0.93-1.06)	≥0.05
Winter	74,553	4.21	1.08 (1.02-1.16)	<0.05

[1] The combination of calf sex and birth number, [2] SM = male sex in singleton births, [3] SF = female sex in singleton births, [4] TMM = male pairs in twin births, [5] TMF = mixed-sex pairs in twin births, and [6] TFF = female pairs in twin births

tended to be at higher risk of being stillborn than female calves [OR (95% CI) = 0.51 (0.44-0.58)]. The risk was also considerably higher when twin births of male pairs and mixed-sex pairs occurred [ORs (95% CI) = 1.88 and 1.51 for TMM and TMF vs. SM, respectively].

The frequency of stillbirth differed according to the year of calving but there was no evidence of consistent phenotypic trends for stillbirths from 2000 to 2009. Increased first calving age resulted in a significantly lower incidence of stillbirth. Effect of inbreeding on stillbirth incidence was not significant (P≥0.05).

Dystocia frequency and its risk factors

The effects of risk factors on calving difficulty are presented in Table 3. Of 342,035 calvings, 86.9, 5.4, 6.37, 1.22 and 0.1% of the calving events were grouped into CES1, CES2, CES3, CES4 and CES5, respectively. Dystocia scores of ≥3 were more common for younger than for older cows (P<0.01). Age at first calving significantly (P<0.01) decreased the probability of calving difficulty. The probability of dystocia was higher in dams giving birth to male than female calves. The risk of dystocia was highest when twin births of male pairs occurred (P<0.01). First calving was associated with more difficult calving than other parities (P<0.01). Maternal inbreeding was not associated with calving difficulty (P≥0.05).

Table 3: Estimated LSMEANS (standard errors) of the effect of parity and the combination of calf sex and birth number (SST) on calving difficulty

SST[1]	Calving difficulty	Parity	Calving difficulty
SM[2]	1.23 (0.003)[c]	Ln=1	1.36 (0.005)[a]
SF[3]	1.14 (0.003)[a]	Ln=2	1.21 (0.005)[b]
TMM[4]	1.29 (0.012)[b]	Ln=3	1.20 (0.005)[b]
TMF[5]	1.26 (0.011)[b]	Ln=4	1.19 (0.005)[b]
TFF[6]	1.24 (.013)[bc]	Ln≥5	1.20 (0.005)[b]
Mean (±SD)	1.22 (0.62)	Mean (±SD)	1.22 (0.62)

[1] The combination of calf sex and birth number, [2] SM = male sex in singleton births, [3] SF = female sex in singleton births, [4] TMM = male pairs in twin births, [5] TMF = mixed-sex pairs in twin births, and [6] TFF = female pairs in twin births. [a, b] within columns, the least square means with a common superscript do not differ significantly (P≥0.05)

Discussion

The mean level of inbreeding was 0.7%, which is smaller than the values in published studies carried out around the world (Adamec *et al*., 2006; González-Recio *et al*., 2007; McParland *et al*., 2007). Tohidi *et al*. (2000) reported that mean level of inbreeding in a sample of Holstein herds in Iran was 0.18%, and 0.35% of inbreed animals had inbreeding coefficients greater than 12.5%. One possible explanation for the low level of inbreeding is incomplete pedigrees. More than 60% of Holstein cows of Iran are inseminated by sperm of proven bulls, imported from the USA and Canada. Pedigree information related to imported semen is limited. The degree of underestimation depends on the amount of missing pedigree information.

Of 365,021 calvings, 2.7% were twin birth which is close to the value reported for a sample of Holstein cows in Isfahan (2.66%; Atashi, 2011) and British dairy cattle (2.5%; Eddy *et al*., 1991), but smaller than the twinning rates in Dutch Friesian and Holstein cattle (3.2%; Nielen *et al*., 1989). Seasonal effects on twinning have been reported for dairy cattle peaking from April to September in the Netherlands (Nielen *et al*., 1989) and from May to July in the USA (Cady and Van Vleck, 1978). Johanson *et al*. (2001) reported that twinning rate was highest during the months leading up to the summer (5.88%) and lowest in winter (4.23%). The greater twinning incidence in the present investigation was associated with conception occurring from mid-summer to mid-autumn and could be a consequence of increased frequency of multiple ovulations due to summer heat stress (Sartori *et al*., 2002).

The percentages of twin births at parities 1, 2, 3, 4 and ≥5 were 0.72, 3.4, 4.51, 4.70 and 5.23%, respectively. Johanson *et al*. (2001) reported that twinning tended to increase with parity of the cow where a pronounced increase from 1.63% at the first parity to 5.22% at the second parity was recorded.

The results showed that twinning rate

was decreased from 0.75% in 2000 to 0.54% in 2009 for primiparous, and increased from 2.35% in 2000 to 3.79% in 2009 for multiparous cows. Silva del Rıo *et al*. (2007) reported that twinning rate was increased from 4.02 and 5.12% in 1996 to 5.75 and 6.71% in 2004 for primiparous and multiparous Holstein dairy cows, respectively.

Of 305,057 calvings, 3.78% were stillborn calves, while the incidence of stillbirth by herd ranged from 0.27 to 9.97%. In a sample of Iranian Holstein cows in Isfahan, the incidence of stillbirth was 6% (Atashi, 2011). According to Meyer *et al*. (2000) and Bicalho *et al*. (2007), about 7% of the Holstein calves born in the USA died within 48 h of birth. The incidence of stillbirth for primiparous and multiparous cows was 5.40 and 2.65%, respectively. Stillbirth incidence in the second and later lactations drops to half or less than that observed for the first lactation. Meyer *et al*. (2000) reported significant differences in the stillbirth percentage between primiparous (11.0%) and multiparous (5.7%) cows. Stillbirth rate is highest in first-calving cows, partly because of a disproportion between size of calf and pelvic area, which causes a difficult calving and increases stillbirth incidence (Bicalho *et al*., 2007).

The greatest stillbirth frequency was observed when parturition occurred in winter. Silva del Rıo *et al*. (2007) reported greater calf mortality during the cold seasons compared with warmer seasons.

The average incidence of stillbirth for singleton and twin births was 3.73 and 5.93%, respectively. The reason may be shorter gestation length and greater incidence of calving difficulty for cows calving twins as the cause of decreased perinatal viability (Berry *et al*., 2007). We found that after single birth, male calves tend to be at higher risk of being stillborn than female calves. This effect may partly be explained by the fact that male calves are in general heavier than female calves.

The frequency of stillbirth differed greatly according to the calving year but there was no evidence of consistent phenotypic trends for stillbirths from 2000 to 2009. Berglund *et al*. (2003) demonstrated that during the past 20 years an increase from about 6 to 10.3% occurred in the incidence of stillbirth in the USA.

Increased age at the 1st calving resulted in a lower incidence of stillbirth. This is expected because increased age would be associated with greater size at the 1st calving.

Effect of inbreeding on stillbirth incidence was non significant. Adamec *et al*. (2006) reported that incidence of stillbirths in first parity births increased by 0.25 and 0.20% for male and female calves per 1% increase in inbreeding. Thompson *et al*. (2000) reported that inbreeding affected phenotypic performance for reproduction in young animals with moderate effect in mature animals. Maternal inbreeding has been shown to reduce growth rates of heifers, potentially increasing stillbirth in first parity births (Thompson *et al*., 2000). McParland *et al*. (2007) demonstrated that calf inbreeding significantly affected perinatal mortality in Charolais, Simmental, and Hereford, and dam inbreeding significantly affected perinatal mortality in Limousin and Hereford.

Of 342,035 calvings, 86.9, 5.4, 6.37, 1.22 and 0.1% were grouped into CES1, CES2, CES3, CES4 and CES5, respectively. Adamec *et al*. (2006) reported that 82.32, 7.3, 6.6, 2.3 and 1.3% of 218,183 calvings were grouped into CES1, CES2, CES3, CES4 and CES5, respectively.

We found that dystocia scores of ≥3 were more common for younger than for older cows, partly because of a disproportion between size of calf and pelvic area. The probability of dystocia was higher in dams when giving birth to male than giving birth to female calves. This effect may be partly explained by the fact that male calves are in general heavier than females. Our results demonstrated that the risk of dystocia was highest when twin births of male pairs occur, the reason may be shorter gestation length.

In this study, maternal inbreeding was not associated with calving difficulty. Maternal inbreeding has been shown to reduce growth rates of heifers, potentially increasing calving difficulty (Adamec *et al*., 2006). González-Recio *et al*. (2007) reported that inbred cows showed impaired fertility and tended to have more difficult calvings than low/or non-inbred cows, but did not increase dystocia incidence. McParland *et al*. (2007) reported that dam and calf inbreeding did not affect dystocia in Charolais, Limousin, Simmental and Hereford beef cattle. Dam inbreeding level, but not calf inbreeding level, was associated with increased dystocia in first parity Angus dams (McParland *et al*., 2007). Rokouei *et al*. (2010) reported a significant effect of inbreeding on calving interval, age at first calving and calving ease in Holstein dairy cows of Iran.

Although inbreeding may be a factor in calving problems, in this study maternal inbreeding had no effect on twinning, dystocia and stillbirth incidence. The incidence of dystocia and stillbirth was impacted by herd, suggesting that management practice differences among farms may influence dystocia and stillbirth rates. Herd managers should review calving procedures with their veterinarians to assure that proper timing and calving assistance techniques are used when providing assistance during parturition. First parity cows were at the highest risk of having dystocia and stillbirths, therefore a suitable managerial practice for reducing the incidence of dystocia and stillbirth may utilize sire and daughter calving ease information when selecting sires for breeding cows and especially the heifers. Alternatively, an improved heifer feeding program should be implemented to ensure optimal heifer size at breeding.

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