Factors Affecting Days Open in Holstein Dairy Cattle in Khorasan Razavi Province, Iran; A Cox Proportional Hazard Model

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Abstract: We conducted a retrospective cohort study to investigate factors influencing the reproductive performance of dairy cattle in Khorosan Razavi province, Iran. Ten dairy herds, located within a 45 km radius of the city of Mashhad in Khorosan Razavi province took part in the study. Complete lactation records were collected for cows that calved between 21st March 2006 and 20th March 2007. Each cow was followed until the end of the study on 21st October 2007 or until the date of leaving the herd, either by culling, sale, or death. Median days open was 123 days (range 28-430 days). Cox proportional hazard models with and without a herd level frailty term were used to identify and quantify the effect of factors influencing days open. Parity and the presence of uterine infection, cystic ovarian disease, mastitis and lameness were positively associated with days open. The proportion of variance explained at the herd level was 0.33% suggesting that the herds that participated in this study were relatively homogenous in the distribution of unmeasured herd-level factors influencing days open. This study has provided starting point for defining benchmark estimates of reproductive performance in dairy herds in this area of Iran. Quantifying the effect of disease on reproductive performance provides a means for ranking disorders in terms of their effect on fertility, allowing intervention strategies designed to optimise herd health and production to be further refined.

Key words: Cox proportional hazard model, frailty term, dairy cows, holstein dairy cattle

INTRODUCTION

In seasonal and non-seasonal dairy production systems the predictable production of milk and young stock is dependent on calving pattern and for this reason; reproductive performance is a key determinant of profitability (Farin *et al.*, 1994).

Days open is routinely used to assess reproductive performance and to make economic decision in dairy herds (Arthur *et al.*, 2001; Farin *et al.*, 1994; Harman *et al.*, 1996a). Knowledge of factors influencing the fertility of individual cows is important so that they can be managed, where appropriate. Multivariable regression analyses of accumulated reproductive data are particularly useful in this respect, since they allow the effect of key determinants of reproductive success to be quantified while, controlling for the effect of known confounders.

Survival analysis (Collett, 2003; Kleinbaum and Klein, 2005; Klein and Moeschberger, 2003) provides a number of advantages over other regression techniques when describing and quantifying the effect of factors influencing days open in dairy cattle. The primary advantage of survival analysis is that it can account for censored observations, such as cows that are sold or culled throughout the breeding period for reasons apart from reproductive failure, or bred cows where the outcome of breeding is unknown at the end of the study observation period. Accounting for censored data provides less biased estimates of the impact of factors affecting overall reproductive performance (Farin *et al.*, 1994; Lee *et al.*, 1989; Del *et al.*, 2005, 2006) and for this reason, survival analysis is the recommended method for analysis of dairy cow reproductive data (Annual Report of Cooperative Regional Research, 2003).

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Most studies that have used survival analysis to investigate factors influencing fertility in dairy cattle have evaluated the effect of variables such as parity and milk yield on days open (Farin *et al*., 1994; Eicker *et al*., 1996; Harman *et al*., 1996a, b; Meadows *et al*., 2006; Melendez and Pinedo, 2007). Parity and milk yield are variables routinely recorded for all cows in herds participating in dairy herd improvement schemes. Widespread participation in dairy herd improvements schemes means that data can be gathered with relative ease, allowing greater numbers of herds to be enrolled in observational studies, which in turn increases study power and increases the precision of estimated effects. The disadvantage of this approach is that routinely recorded variables are usually indirectly, rather than directly, associated with reproductive performance. For example, a negative association between parity and days open may arise because the risk of certain diseases (e.g., retained foetal membranes and uterine metritis) increases with parity and it is the presence of disease that directly impacts on reproductive performance, as opposed to parity alone. This being the case, observational studies that investigate the effect of factors directly associated with reproductive failure, such as disease (Lee *et al*., 1989; Harman *et al*., 1996a, b) are likely to provide information of greater use to herd managers and their veterinarians in terms of understanding factors directly influencing reproductive performance.

In any observational study, it is likely that factors associated with reproduction may not be measured or easily quantified. These factors are often present at multiple levels, for example nutrition, calving hygiene and housing at the herd level, genetics (intrinsic fertility) at the cow level and the presence or absence of disease events at the individual lactation level. Shared frailty models for survival analysis provide a means for accounting for these unmeasured (latent) variables and are analogous to random effects models in linear regression (Gutierrez, 2002). Frailty models have been applied to both parametric (Schnier *et al*., 2004; Meadows *et al*., 2007) and semiparametric survival models of dairy cow fertility (Maizon *et al*., 2004). Shared frailty models account for latent variables that operate at multiple levels above the unit of observation such as lactations nested within cows, cows within herds and herds within geographic regions (Dohoo *et al*., 2001; Gutierrez, 2002). This approach allows investigators to determine the relative contribution of individual and group level effects on fertility, which in turn allows herd managers to better target strategies designed to enhance reproductive performance (Dohoo *et al*., 2001).

Although, factors influencing the fertility of dairy cattle have been well documented in most of the major dairy producing countries throughout the world it is not clear if the findings from these studies can be applied to Iran. To address this knowledge gap, we present a study of factors influencing fertility in 10 Holstein Friesian dairy herds in Khorasan Razavi province, in the north east of Iran. Survival analyses, specifically Cox proportional hazard models, have been used to identify factors influencing days open.

MATERIALS AND METHODS

Data for this study were derived from commercial dairy farms located within a 45 km radius of the central business district of Mashhad, the capital city of the province of Khorasan Razavi in The Islamic Republic of Iran (Fig. 1). The climate in this area is characterized by cold dry winters (temperature range -3-10°C) and hot dry summers (temperature range 17-33°C). Commercial dairy herds in this area are comprised predominantly of Holstein-Friesian cattle that calve throughout the year and re-bred using artificial insemination. Cows are housed in open-shed barns and milked 3 times daily. Herds are fed on total mixed rations; diets are based primarily on corn silage, alfalfa hay and concentrates.

This was a retrospective cohort study. The source population was comprised of commercial dairy herds registered with the Mashhad Farmers' Union. Eligible herds were those that had established systems (either hand written or computerised) to record herd and individual animal event details. Ten herds on the Mashhad Farmers' Union register met the eligibility criteria and agreed to take part in the study.

In each of the study herds cows were rebred after calving using artificial insemination carried out by a contract inseminator. Estrous detection was conducted on a time-planned observation schedule and diagnosis of pregnancy was carried out using ultrasonography between 30 and 35 days after breeding in eight herds and by rectal palpation between 42 and 50 days after breeding in the remaining 2 herds. Four of the 10 herds had a full time on-staff veterinarian; the remainder employed a private veterinary practitioner who was consulted on a regular basis for the purpose of managing herd health. Median herd size was 280 lactating cows (range 120-610 cows). Voluntary waiting periods ranged between 34 and 60 days. Mean parity was 2.8 (range 1-9) and culled cows were replaced with heifers either reared on farm, or purchased from other herds.

In each of the study, herds complete lactation records were collected for cows that calved between 21 March 2006 and 20 March 2007 (inclusive) and received at least one insemination following calving.
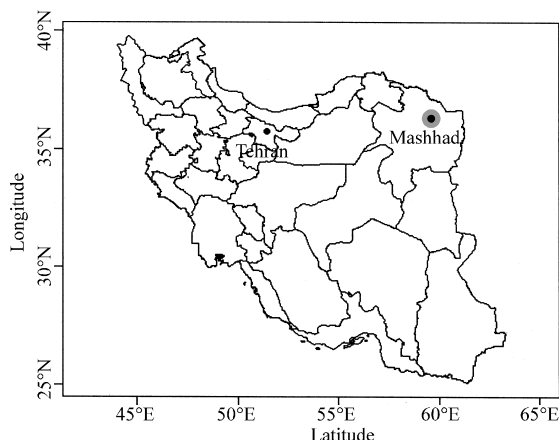
Fig. 1: Map of the provinces of the Islamic Republic of Iran showing the location of the cities of Tehran and Mashhad (Khorasan Razavi province). Farms that participated in this study were located within 45 km of the Mashhad city boundaries (indicated by the gray shaded aear on the map)

Sample size calculations indicated that at least 1, 110 lactation records were required to be 95% certain that our estimate of days open was within 5 days of the true population value (Cleves *et al*., 2008). The number of lactation records selected from each of the 10 herds was proportional to median lactating herd size. Thus, if the median lactating herd size of farm A was 20% of the sum of median herd sizes across all herds then at least 220 lactation records (0.20×1.100) were selected from farm A.

A systematic random sampling approach was used to select lactation records from each herd. A sampling interval (k) was calculated by dividing the total number of lactations that commenced during the recruitment period (21st March 2006 and 20th March 2007) by the required herd sample size. A random number between 1 and k was selected using a random number generator and the kth calving event that occurred after 21st March 2006 and every kth calving event after that selected for inclusion in the study. A total of 1,988 lactation records comprised the study data set.

For each lactation record we retrieved the individual cow identifier, parity, the length of the voluntary waiting period (in days), calving date, season of calving (fall: September to November; winter: December to February; spring: March to May and summer: June to August), date of insemination event (s) and mean milk yield (L) for the first 4 herd tests (that is, up to 120 days after calving). The presence or absence of the following health disorders that occurred before the estimated date of conception or censoring (up to 120 days in milk) were recorded: stillbirth, dystocia, retained fetal membranes,

milk fever, uterine infection, cystic ovarian disease, clinical mastitis and lameness. Disease diagnoses were made by the on-staff veterinarian in 4 herds and by the on-call veterinarian in the remaining 6 herds. The diagnostic criteria used for each disorder are shown in Table 1. Each cow was followed up until the end of the study on 21st October 2007 or until the date an individual left the herd, either by culling, sale or death, whichever occurred first.

For each lactation record a date of conception was determined as follows. For cows with a calving event between 21st March 2006 and 20th March 2007 that was followed by a 2nd calving event up to 21st October 2007, the date of conception was defined as the service date that occurred within 7 days of the 2nd calving event less 281 days. For all other lactations the date of conception was determined on the basis of pregnancy testing. For these lactations the date of conception was defined as the date of their last recorded breeding event, unless specified otherwise in the notes recorded by the herd veterinarian at the time of pregnancy testing. Cows without a subsequent calving event and cows without a confirmed pregnancy diagnosis until the end of study (21st October, 2007) were censored on the date of their last recorded breeding event.

All analyses were carried out using Stata Statistical Software, release 8.0 (Stata Corporation, College Station, Texas, USA). Parity was coded as a categorical variable comprised of 4 levels: 1, 2, 3 and $4. Mean lactation yield in the first 120 days was coded as a categorical variable of 5 levels: <20, 20-30, 30-40, 40-50 and >50 L.

The association between each explanatory variable thought to influence days open was tested using the log rank test. Each explanatory variable was categorised into 2 or more levels. Kaplan-Meier survival curves for each level of an explanatory variable were plotted and the homogeneity of the curves between levels tested using the log rank statistic. Explanatory variables that showed an association with days open (that is, a difference in the Kaplan-Meier survival curves that was significant at p<0.20) were selected for inclusion in the multivariate analysis.

We used a Cox proportional hazard model to quantify the effect of each of the prescribed explanatory variables on days open. The general form of the Cox proportion of hazard model is:

$$h(t,X)=h_0(t)e^{\sum_{i=1}^{p}\beta_i X_i} \qquad (1)$$

This model gives an expression for the hazard at time t for an individual with a given specification of a set of explanatory variables denoted by the bold X. That is, the bold X represents a collection (sometimes called a vector) of predictor variables that is being modeled to predict an individual's hazard.

Table 1: Definitions of disease diagnoses

Category	Diagnosis	Comments
Calving-associated disorders	Dystocia still birth	Any assistance provided for delivery of the calf dead calf at birth after full term of gestation
Metabolic disorders	Milk fever	Characteristic clinical signs and response to therapy
Reproductive tract disorders	Retained foetal membranes	Membranes defined as retained if not passed with 24 h after calving
	Uterine infections	Purulent discharge on vaginal examination
	Cystic ovarian disease	History of abnormal oestrus cycles accompanied by abnormal findings on ovarian palpation
Udder disorders	Mastitis	Clinical mastitis or elevated individual cow somatic cell count warranting, in the herd manager's opinion, intramammary therapy
Locomotor disorders	Lameness	Any condition affecting the foot (footrot, solar bruising, foot abscess)

The Cox model formula says that the hazard at time t is the product of 2 quantities. The 1st of these, h_0 (t), is called the baseline hazard function. The 2nd quantity is the exponential expression e to the linear sum of $\$_i X_i$, where the sum is over the p explanatory X variables.

In Cox proportional hazards model coefficients represent, the expected change in hazard for 1 unit changes in a predictor.

To select those explanatory variables that best explained days open a backward stepwise approach was used. The significance of each explanatory variable in the model was tested using the Wald test. Explanatory variables that were not statistically significant were removed from the model one at a time, beginning with the least significant, until the estimated regression coefficients for all retained variables were significant at an " level of <0.05. Proportional hazard assumption was tested and extended Cox model using time varying covariate was generated. Finally, a shared frailty term based on the gamma distribution was included to account for herd-level effects on days open.

RESULTS AND DISCUSSION

Descriptive statistics for variable including in this study are provided in Table 2 and 3. Fourteen percent (282 of 1988) of cows were censored during the study. Median days open for cows identified as pregnant was 123 days (range 28-430 days).

Both model with and without frailty term at herd level chose the same range of significant variable.

The final model (Table 4) included parity and the following disease conditions: uterine infection, cystic ovarian disease, lameness and mastitis

On average, cows in fourth or higher parity had a 14% lower conception rate compared with parity 1 cows (p = 0.03).

Cows with uterine infections had 37% lower conception rate (HR = 0.63; 95% CI = 0.56-0.70). Cows with lameness had 22% lower conception rate (HR = 0.78 (0.63-0.96) and cows with mastitis had 16% lower conception rate (HR = 0.84 (0.72-0.99) than those of cows that were free of these disorders.

Cystic ovarian was time dependent variable and negative effect of this disorder on fertility decreased if it occurs later after calving (e.g., if cystic ovarian disease occur in day 50 after calving hazard ratio will be $e^{-1.2054 + 0.0041 \times 50} = 0.37$. it means that cows with occurrence of cystic ovarian disease at day 50 had 63% lower conception rate than those of cows that were free of this disorder. And if cystic ovarian disease occur in day 120 after calving hazard ratio will be $e^{-1.2054 + 0.0041 \times 120} = 0.49$ that indicate cows with occurrence of cystic ovarian disease at day 120 had 51% lower conception rate than those of cows that were free of this disorder.

The proportion of variance explained at the herd level was 0.33%. Random selection of farms for this study was not possible as a result of our requirement for herd managers to observe and record detailed event information for all cows within their herds. Average herd test yield for the first 120 days in milk was 37 kg, equivalent to a 305 day milk yield of 8.235 kg, >the 7. 320 L average estimated for commercial dairy herds in this area of Iran (Anonymous, 2003). Purposive selection therefore appears to have in a set of higher-producing herds compared with the Mashhad Farmers' Union average (Anonymous, 2003). This bias may therefore, limit our ability to generalize our descriptive analyses of reproductive performance to other Iranian dairy herds. Due to the mostly physiological effect of each of the explanatory variables on the outcome (Elwood, 1998), we believe that the findings of our multivariate analyses can be applied to the general population of Iranian dairy herds.

Median days open for the 10 herds that participated in this study was 123 days, similar to the median of 124 days reported by both Farin *et al*. (1994), in a study of 2000, Holstein Friesian cows in North Carolina and Silva *et al*. (1992), in a study of Holstein Friesian cows in Florida, USA. A median of 123 days open is equivalent to a calving interval of 12-13 months, cited as optimal for dairy herds by Radostitis and Blood (2001). The true calving interval in these herds is likely to be >12-13 months given that our estimate of 123 days is based on cows where the date of conception was actually known as well as cows with censored observations.

Table 2: Descriptive statistics of continuous variables in the studied Iranian dairy herds (n =1988 complete lactation records from 10 herds)

Variable	n	Mean (SD)	Median (Q1, Q3)	Min, Max
Voluntary waiting period (days)	1988	44.0 (7.3)	45 (40.500)	34.600
Parity	1988	2.4 (1.4)	2 (1.300)	1.900
Days open	1988	152.0 (0.0)	123 (82.193)	28.430
Milk yield at day 120 (L)	1988	37.0 (8.0)	37 (32.420)	10.660

Table 3: Descriptive statistics of categorical variables in the studied Iranian dairy herds (n = 1989 complete lactation records from 10 herds)

Variable	Cases	Non-cases	Incidence risk[a]
Dystocia	176	1812	8.9 (7.7-10.2)
Stillbirth	62	1926	3.1 (2.4-4.00)
Retained placenta	130	1858	6.5 (5.5-7.70)
Milk fever	40	1948	2.0 (1.5-2.70)
Uterine infections	594	1394	30.0 (28-3200)
Cystic ovarian disease	94	1894	4.7 (3.9-5.80)
Lameness	122	1866	6.1 (5.2-7.30)
Mastitis	219	1769	11.0 (9.7-12.5)

A factor contributing to days open at the herd level in this study may have been the length of the voluntary waiting period (that is, the number of days after calving permitted to elapse before breeding commences). For these herds mean voluntary waiting period was 44 days (range 34-60), >53 days reported by Caraviello *et al.* (2006) in a study of 103 US herds and 56 days reported by DeJarnette *et al.* (2007) in a study of 583 dairy herds throughout the USA.

Our estimates of disease frequency (Table 3) were broadly similar to those reported in Pennsylvania, USA (Lee *et al.*, 1989), Michigan, USA (Kaneene and Hurd, 1990), Switzerland (Frei *et al.*, 1997) and Australia (Stevenson, 2000). The relatively low incidence of milk fever in our study may have been due to the effect of preventive treatments given at the time of calving (e.g., oral calcium chloride) or due to the effect culling, bearing in mind that cows that took part in this study were those that received at least one insemination event following the date of calving. The relatively high incidence risk of uterine infection (30 cases /100 cows at risk) was most likely due to intensive surveillance for this disorder arising from routine post natal reproductive examinations.

We identified no significant association between milk yield in the first 120 days of lactation and days open. The effect of milk yield on days open in previous studies of dairy cow fertility has been variable. High milk yields were associated with a reduced hazard of pregnancy in a retrospective study of 2000 Holstein Friesian cows in North Carolina, USA (Farin *et al.*, 1994). Meadows *et al.* (2006) reported a similar effect in a fixed-effects model of factors influencing hazard of pregnancy in 11,398 Holstein Friesian cows in Ohio, USA. When this model was extended to include milk yield as a time varying covariate this association was no longer present. Harman *et al.* (1996b), in an observational study of 44,450 cows in

Finland, found that high milk yields were associated with an increase in days open, but only in those cows that produced high fat content milk. Rajala-Schultz and Frazer (2003), in a study of 1772 herds in Ohio, USA, identified a negative association between milk yield and first service conception rate and a positive association between milk yield and calving interval (increased milk yield, shorter calving interval). The interpretation provided by these authors was that high producing herds were able to compensate for the negative effects that high milk production has on fertility by aggressive reproductive management. This explanation would be consistent with the findings reported here.

We didn't find relationship between calving season and days open. Eicker *et al.* (1996) found that cows, which calved during the spring (between March and May) had lower conception rates compared with those that calved in winter (December to February). In the study of Meadows *et al.* (2006) spring and summer calvers had reduced fertility compared to those calving in the winter. Harman *et al.* (1996a), on the other hand, found that the fertility of winter calving cows was lower, compared with cows that calved at other times of the year. The variable effect of calving season on fertility is noteworthy and is likely to be due to a number of factors operating at the herd level including length of voluntary waiting period, temperature, temperature range and photoperiod.

We identified a direct relationship between parity and days open, similar to findings reported elsewhere (Eicker *et al.*, 1996; Harman *et al.*, 1996b; Meadows *et al.*, 2006). Eicker *et al.* (1996) found that cows of parity 3 or greater had, on average, an 8% lower conception rates compared with parity 1 cows. Meadows *et al.* (2006), using a Cox proportional hazards regression model with milk yield as a time varying covariate, found that cows of parity 2, 3, 4 and $5 had 0.95, 0.94, 0.90 and 0.82 times the daily hazard of conception compared with parity 1 cows. Our findings indicate that reproductive management programs should target multipara-perhaps in the form of routine post partum examinations so that reproductive tract disorders, if they are identified, can be identified and aggressively treated.

A number of studies have identified a detrimental effect of disease on reproductive performance (Lee *et al.*, 1989; Eicker *et al.*, 1996; Harman *et al.*, 1996; Loeffler *et al.*, 1999; Maizon *et al.*, 2004), similar to the findings reported here. In our study, cystic ovarian

Table 4: Factors associated with days open in 1988 Holstein dairy cows calving between 21st March 2006 and 20th March 2007

Variables	No. frailty term			Herd level frailty		
	$ (SE)	p-value	Hazard ratio (95% CI)	$ (SE)	p-value	Hazard ratio (95% CI)
Herd						
1	0	-	-	-	-	-
2	0.3961 (0.1141)	<0.01	1.49 (1.19-1.86)	-	-	-
3	0.1501 (0.1083)	0.17	1.16 (0.94-1.44)	-	-	-
4	-0.1808 (0.1180)	0.13	0.83 (0.66-1.05)	-	-	-
5	0.1886 (0.0724)	0.01	1.21 (1.05-1.40)	-	-	-
6	-0.0107 (0.1011)	0.92	0.99 (0.81-1.21)	-	-	-
7	0.0233 (0.1183)	0.84	1.02 (0.81-1.29)	-	-	-
8	-0.0159 (0.1258)	0.90	0.98 (0.81-1.21)	-	-	-
9	-0.0078 (01017)	0.94	0.99 (0.81-1.21)	-	-	-
10	0.0511 (0.1225)	0.68	1.05 (0.83-1.34)	-	-	-
Parity						
1	0	-	1	0	-	1
2	-0.0484 (0.0652)	0.46	0.95 (0.84-1.08)	-0.0530 (0.0651)	0.42	0.95 (0.83-1.08)
3	-0.0976 (0.0680)	0.15	0.91 (0.79-1.04)	-0.1040 (0.0678)	0.13	0.90 (0.79-1.03)
4	-0.1540 (0.0696)	0.03	0.86 (0.75-0.98)	-0.1550 (0.0689)	0.02	0.86 (0.75-0.98)
Diseases						
Uterine infections	-0.4825 (0.0575)	<0.01	0.62 (0.55-0.69)	-0.4677 (0.0562)	<0.01	0.63 (0.56-0.70)
Lameness	-0.2659 (0.1067)	0.01	0.77 (0.62-0.94)	-0.2477 (0.1061)	0.02	0.78 (0.63-0.96)
Mastitis	-0.1857 (0.0819)	0.02	0.83 (0.71-0.98)	-0.1686 (0.0807)	0.04	0.84 (0.72-0.99)
Cystic ovarian[a]	-1.2054 (0.2606)	<0.01	0.30 (0.18-0.50)	-1.1733 (0.2603)	<0.01	0.31 (0.19-0.52)
Cystic ovarian*t[a]	0.0041 (0.0014)	<0.01	1.00 (1.00-1.01)	0.0041 (0.0014)	<0.01	1.00 (1.00-1.01)
Theta[b]	-	-	-	0.0110 (0.0088)[c]	-	-

[a]Interpretation: Controlling for the effect of herd, parity and other disease conditions, conception rate for cows with a diagnosis of Cystic ovarian is $e^{-1.2054+0.0041*t}$ time of cows without a diagnosis of Cystic ovarian disease where, t is time of disease occurrence after calving; [b]Variance of the unobserved frailty parameter; [c]p = 0.006 for likelihood ratio test of H_0: Theta = 2

disease had the greatest impact on days open followed by uterine infection, mastitis and lameness. Eicker *et al.* (1996) found that cystic ovarian disease, metritis and retained placenta reduced conception rates by 21, 15 and 14%, respectively. Lee *et al.* (1989) found that retained fetal membranes, metritis with and without systemic signs, cystic ovarian disease and lameness decreased the daily hazard of conception by factors of 0.66, 0.83, 0.70, 0.70 and 0.69, respectively.

In our study, effect of Cystic ovarian disease was time dependent and its negative effect decreased if cystic ovarian disease occurs later postpartum. Maizon *et al.* (2004) in an observational study of factors associated with days open in 23927 Swedish red and white dairy cattle found that if ovulatory dysfunction occurs during first 45 days after calving it can decrease hazard of conception by 36% and this amount of decrease was not observed if it occurs after 45 days.

Although, the herd-level frailty term was significant in our model (Table 4), its inclusion in the model had little impact on the magnitude of the covariates estimated for each of the fixed effects. These findings indicate that the herds that participated in our study were homogenous in the distribution of unmeasured, herd-level factors influencing days open, a conclusion supported by the similarity of the Kaplan-Meier survival curves of days open for each participant herd (Fig. 2). This explanation is reasonable, given that all of the herds that participated in this study had a similar system of management and all had relatively high numbers of farm staff relative to herd size

(n = 1.2/100 lactating cows). In agreement with other authors (Dohoo *et al.*, 2001) these findings also indicate that variation in days open in dairy cattle is largely driven by factors operating at either the individual cow or individual lactation level. Extension of this study, to accumulate multiple lactation records per cow would allow the relative contribution of individual cow and lactation level effects on reproductive performance in this area of Iran to be defined in greater detail.
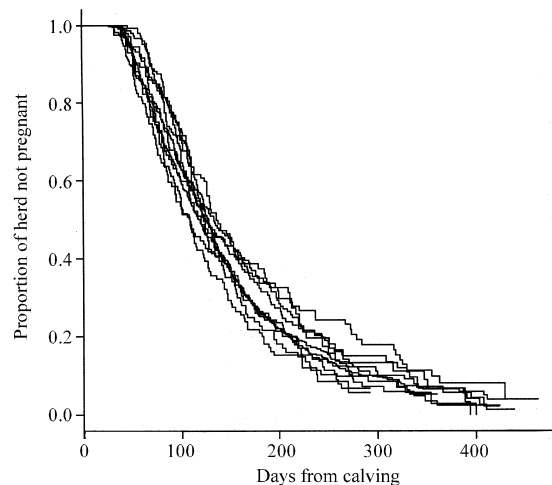

Fig. 2: Kaplan-Meier survival curves showing the proportion of each of 10 herds in Khorosan Razavi province, Iran, not pregnant as a function of days from calving

CONCLUSION

This study has provided a useful starting point in terms of providing benchmark estimates of reproductive performance in dairy herds in this area of Iran. Parity and the presence of disease in the 1st 120 days of lactation were associated with days open in this group of herds. With these factors identified, the challenge now is to implement interventions to minimise their negative effect on reproductive performance. Older stock should be targeted for routine post natal reproductive examinations so that treatment can be applied, where necessary. Equally important in this regard is attention to heifer replacement management, which increases the capacity for herd managers to replace older, infertile cows with well-grown young stock. Although, lameness and mastitis are not direct causes of infertility, both conditions were significantly associated with days open in this group of herds. Estimates of the economic effect of each of these conditions should take this into account.

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