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Factors affecting reproductive performance of dairy cows in a pasture-based, automatic milking system research farm: a retrospective single cohort study

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Abstract. A retrospective single cohort study was conducted to identify production and health factors associated with reproductive performance in a pasture-based automatic milking system research farm. The calving system of this herd shifted from split calving to year round calving gradually during the study period. Data from 365 cows with 798 lactations were analysed in the study. Reproductive outcome variables of interest were intervals from calving to first oestrus, to first insemination, and to conception as well as number of inseminations per conception, probability of submission for insemination by 80 days in milk, probability of conception by 100 days in milk and probability of conception at first insemination. Production factors (milk yield and its composition; milking frequency), record of periparturient disease, parity and season of calving were considered as predictor variables. The associations between predictor and outcome variables were assessed by multivariable linear regression, logistic regression and survival analyses for quantitative, binary and time-to-event outcomes, respectively. Average milk yield and milking frequency during 100 days in milk were not significantly associated with any of the reproductive measures. The likelihood of conception by 100 days in milk decreased gradually with year of automatic milking systems commissioning. Cows calved in autumn were 43% (hazard ratio: 1.43, $P < 0.05$) more likely to conceive compared to cows that calved in summer. Multiparous cows were more likely ($P < 0.05$) to be recorded for oestrus compared to primiparous cows. Twinning was negatively associated with the reproductive outcomes measured in the automatic milking systems research herd. Milk yield and milking frequency during 100 days in milk had no effect on reproductive measures in the pasture-based automatic milking system research herd.

Key words: Reproduction, Automatic Milking System, Pasture-Based System

Introduction

The introductions of automatic milking systems are one of the most significant technological changes in the dairy industry since the late 19th century. In Europe, automatic milking systems have been commercially available since 1992. Since then, the number of automatic milking system units installed throughout the world has increased steadily. In late 1997, more than 100 farms were using automatic milking systems, and by early 2004, the figure had increased to over 2200 farms (de Koning and Rodenburg, 2004). Currently more than 10,000 farms globally are using automatic milking systems (de Koning, 2011). Automatic milking systems are becoming increasingly popular due to the growing costs of labour, reduced labour availability and increased desire for improved lifestyle in many dairy countries (Rossing et al., 1985). More recently, the ability to manage the herd at an individual level through the improvement of real-time and individualised data has also likely influenced the uptake of this technology in some markets. Automatic milking systems are often described as a completely new way of farming because of the voluntary cow traffic and resultant distribution of milkings throughout the day and night affects most aspects of herd and farm management. For example, cows managed with conventional milking systems are generally inspected for oestrus behaviour at the time of milking. Milking occurs largely without human intervention with automatic milking systems, traditional oestrus detection methods and/or routines require reconsideration.

In intensive housing systems, a number of studies have been conducted to identify the factors influencing reproductive performance in automatic milking systems, but findings are inconclusive. Under research conditions with an individual feeding and management system in an automatic milking systems (regardless of the milking frequency), significant association has been reported with longer interval from calving to first insemination but no effect on nonreturn rate at 56 d after first insemination (Kruip et al., 2002). A previous study (Weiss et al., 2004) reported the neutral effect of milking system (automatic versus conventional) on the interval from calving to conception and resumption of ovarian cyclicity for the cows kept in a naturally ventilated loose housing barn. On the other hand, Dearing et al. (2004) monitored six farms in The Netherlands for up to six months before automatic milking systems installation through until 12 months post installation and showed an increase in interval from calving to conception as well as reduced conception rate. On the other hand, Löf et al. (2007) reported that automatic milking systems herds in Sweden had shorter calving intervals and shorter intervals from calving to first insemination or to conception compared with conventional milking systems herds. It is possible that the combined effect of individual cow performance, management policy (for example, voluntary waiting period, the method of oestrus detection) and herd size are all factors contributing to the different findings.

We are not aware of any studies that have evaluated the reproductive performance of dairy cows in pasture-based automatic milking systems herds. Therefore, we conducted a retrospective single cohort study to investigate the effects of milk yield and milking frequency during early lactation on reproductive performance. In addition, we evaluated effects of a number of other factors (including management and husbandry techniques, cow factors, disease events, season of calving and insemination details) on the reproductive performance of the pasture-based automatic milking systems research herd.

Materials and methods

Farm and animal management

This retrospective single cohort study involved 365 cows with calving dates between 1 May 2006 and 30 April 2011 as mentioned in Talukder et al. (2012). Only cows that had at least one recorded oestrus or insemination event were included in the analyses (*n = 798*). The pasture-based automatic milking systems research herd (Elizabeth Macarthur Agricultural Institute, Camden, NSW, Australia) consisted of 82% (*n = 660*) Holstein-Friesian cows, 16% (*n = 125*) Illawarra and 2% (*n = 13*) crossbreds. From May 2006 to March 2009, all milkings were conducted through two single-box milking units (DeLaval VMS, Tumba, Sweden). In March 2009, a prototype Robotic Rotary (RR; Automatic Milking Rotary AMR™, DeLaval, Tumba, Sweden; Kolbach et al., 2013) was commissioned and milkings were conducted in a combination of VMS and RR as the prototype equipment was co-developed and tested in a pasture-based system. From early February 2011, all milkings were conducted in the RR and in May 2011, the VMS units were removed completely.

During the study period, cows were always subjected to a voluntary waiting period of 50 days in milk during which time observed oestrus events were recorded but cows were not inseminated. The primary oestrus detection method was visual behavioural observation and was aided by the use of a secondary method, KaMar heatmount detectors (Steamboat Springs, CO). The KaMar was applied to the tail head of each cow at day 45 of lactation and was monitored for colour changes (assumed to be caused by mounting behaviour). Cows that were not recorded for oestrus by 60 days in milk, were drafted and checked by the veterinarian and a KaMar was reapplied if it had been lost. Non-cyclers (cows that did not show oestrus signs during the voluntary waiting period) were checked by the veterinarian at 60 to 65 days in milk and synchronized according to the following protocol only when they were diagnosed with ovarian abnormalities (e.g. persistent corpus luteum or follicular cyst). Oestrus was synchronised with 1 ml (100µg) intramuscular injection of gonadorelin acetate (Gonabreed®, Parnell Laboratories Pty Ltd, Alexandria, NSW) followed by 2 ml (500µg) synthetic prostaglandin analogue, cloprostenol sodium (Estrumate®, Schering-Plough Animal Health Limited, Baulkham Hills, NSW) 7 days later. An additional 1 ml Gonabreed injection was administered 48 hours after the Estrumate injection and fixed time insemination was conducted 8 to 24 hours later. Farm staff programmed the system to have cows in oestrus automatically drafted at the dairy; inseminations were conducted twice daily before being released back with the main herd. Pregnancy status was diagnosed by rectal palpation by veterinarians approximately 6 weeks after insemination. When cows were diagnosed not pregnant, they were injected with cloprostenol sodium (Estrumate®, Schering-Plough Animal Health Limited, Baulkham Hills, NSW) and then monitored for oestrus signs as described above. If any cow was diagnosed pregnant to different services, rechecking was performed by a veterinarian to determine the actual date of insemination that resulted in pregnancy. During the study period, a transitional and gradual change in calving pattern was implemented by shifting from split to year round calving. Animals were routinely vaccinated against Clostridia diseases (Ultravac®, Pfizer Animal Health, West Ryde, NSW) and were regularly treated for parasites.

Data management

Individual cow records were maintained electronically on the automatic milking systems or RR support software (VMSClient and DelPro, Tumba, Sweden). In addition to milk production and milking session records, the herd database included the cow identification, date of birth, parity, breed, calving history, date and details of recorded oestrus events, inseminations, dry-off events, periparturient problems (milk fever, retained foetal membrane, assisted calving), and all treatments. Data related to pregnancy diagnosis and any hormonal interventions were also recorded in the support software. Three lactations recorded with abortions and mummified foetuses were excluded from the final dataset.

Cumulative milk yield and average milking frequency from calving to 100 days in milk were calculated using the electronic data captured in the support software, VMS Client (Tumba, Sweden). Average milking frequency from calving to 100 days in milk was calculated using the average daily number of milkings through the first 100 days in milk. Fortnightly herd tests (analysed milk samples) were conducted until January 2011. Herd test samples and production parameter data were submitted to Dairy Express (University of New England, Armidale, New South Wales, 2531). Milk fat and protein percentage data at 100 days in milk were obtained from the closest herd test event. If the date for 100 days in milk was < 15 d from a record for milk solids content, the nearest date was considered. If the 100 d date was >15 and ≤20 d, an average was obtained using the preceding and succeeding recorded data according to the procedure followed by Moss et al. (2002) and when the nearest sample result was >20 days before or after the 100 d date, the data point was considered as a missing value. All data were collated in an electronic spreadsheet (Microsoft Excel 2007) to allow the outcome variables to be calculated.

Outcome variables

The key reproductive performance measurements of interest were: intervals from calving to first oestrus, to first insemination, and to conception as well as number of inseminations per conception, submission for insemination by 80 days in milk (yes/no), conception to first insemination (yes/no) and conception by 100 days in milk (yes/no). Probability of submission for insemination by 80 days in milk describes the proportion of cows that were inseminated by 80 days after calving while probability of conception by 100 days in milk describes the proportion of cows that conceived by 100 days after calving (InCalf, 2007).

Periods from calving to first oestrus, to first insemination, and to conception were measured in days on a continuous scale and number of inseminations per conception was measured as counts. Where a positive pregnancy diagnosis was recorded, the last insemination date was considered as the day of conception, all other insemination dates were assumed to have resulted in no conception. Number of inseminations per conception and the interval from calving to conception were analysed only for the cows that were recorded with a positive pregnancy diagnosis.

Predictor variables

Predictor variables investigated in this study were milk yield, milking frequency, milk fat percentage and milk protein percentage during early lactation (0 to100 days in milk). Additional variables assessed included season of calving and insemination, parity, breed and periparturient diseases: e.g. assisted calving, retained foetal membrane, milk fever, stillborn and twin birth. The predictor variables are described in Table 1[1]. Interval from calving to first oestrus was also tested as a predictor variable in the model to identify its effect on interval from calving to first insemination and to conception.

Statistical analyses

Among the 798 lactations investigated, cows had a positive pregnancy diagnosis for 86% (*n = 686*) lactations and the dataset of those cows were used for analyses of interval from calving to conception, conception to first insemination and number of inseminations per conception. Frequency tables were created for binary outcome variables. All the continuous predictor and outcome variables were initially assessed using descriptive statistics. Continuous predictors were then tested for the linear association with continuous outcome variables; if there was no linear effect, continuous predictors were then categorized into quartiles for easier interpretation. Continuous predictor variables (except the interval from calving to first interval) such as year, season of calving, season of insemination, season of conception, 100 d milk yield, 100 d milking frequency, 100 d milk fat percentage and 100 d milk protein percentage were categorised into quartiles as described in Table 1.

Linear and logistic regression analyses were performed for continuous and binary outcome variables, respectively. Initially, univariable analyses were conducted and variables significant at a *P*-value < 0.25 were selected for multivariable analyses following the procedure reported by Dhand (2009, 2010). Multivariable analyses were performed to identify groups of predictor variables associated with outcome variables. Biologically relevant interactions were subsequently added to the model following forward stepwise selection procedure and variables significant at $P \leq 0.05$ (based on the Wald's test for logistic regression and partial *P* value for linear regression) were included in the model. Multivariable models were built using manual stepwise approach, assisted by the MultiLogistic macro (Dhand 2009). At each step of the multivariable model, all of the variables were tested in the model by excluding them one at a time, whilst all other variables were tested for exclusion in the model by including them in the model one at a time. P-value of 0.05 was used as a cut-off for both entry and exclusion. So if any of the tested variables in the model was not significant, it was removed, and if one of the tested variables not in the model was significant, it was included.

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[1] All tables are located at the end of this document.

Only one measure of milk solids (either fat percentage or protein percentage separately) and milk yield were included in each multivariable model to avoid the problem of collinearity. Parity, calving season, milk yield and milking frequency during the first 100 days of lactation were included in each final model to avoid any confounding effects of these factors according to Gröhn and Rajala-Schultz (2000) and to obtain effect estimates for those variables. Each final model was then tested by linear mixed model (PROC MIXED; SAS Inst. Inc., Cary, NC) for continuous outcome variables, and generalized linear mixed model (PROC GLIMMIX; SAS Inst. Inc., Cary, NC) for binary outcome variables after adjusting for the clustering effect of cow. Assumptions were evaluated using residual diagnostics. Models have been refitted twice by including all current variables as in the final models but first excluding milking yield and then excluding milking frequency for all the reproductive outcome variables evaluated in this study. Intervals from calving to first insemination had skewed distributions, so logarithmic transformation was performed for this variable before conducting the univariable analyses (presented in Appendix A) and the means were back transformed for presentation of results. The back transformed means represent the geometric means.

The associations between the predictor variables and number of inseminations per conception were tested by Poisson regression using GENMOD followed by PROC GLIMMIX (SAS Inst. Inc., Cary, NC) procedure after adjusting for clustering due to cow. Regression coefficient of each predictor was exponentiated for the interval from calving to first insemination and number of inseminations per conception to calculate ratios of geometric mean and counts respectively. Times to reproductive events (i.e. interval from calving to first oestrus and to conception) were tested by survival analyses with Cox’s proportional hazard model (Procedure PHREG; SAS Inst. Inc., Cary, NC) and adjusted for the clustering effect of cow. Hazard ratios (HR) and their confidence intervals (CI) were presented in results. Cows that were not recorded for at least one oestrus by 49 days in milk were considered as right censored for first oestrus event while cows that were not pregnant by 6 weeks before date of death or date of culling or 6 weeks before the last data entry date in the dataset were censored for the conception event.

Results

Only cows that had at least one recorded oestrus or insemination event were included in the analyses (n = 798). We excluded the cows that had no records after calving as they either had died or were culled from the herd during the very early stages of lactation. We speculated that inclusion of cows with at least one oestrus or insemination event caused minimal selection bias.

Descriptive analyses

Descriptive statistics of the continuous outcome variables are presented in Table 2. For binary outcome variables, in 49% (*n = 391*) and 36% (*n = 247*) of lactations, cows were inseminated by 80 days in milk and were pregnant by 100 days in milk respectively while in 50% (*n = 399*) of lactations, cows conceived to the first insemination. Among the 798 lactations investigated, cows had a positive pregnancy diagnosis for 86% (*n = 686*) of lactations and these lactations were used to analyse conception at first insemination, interval from calving to conception and number of inseminations per conception.

Interval from calving to first oestrus

Among the 798 lactations investigated, for 38% (*n = 305*) lactations, the cow had at least one oestrus event by 49 days in milk. Out of the 15 variables listed in Table 1, six (year, season of calving, parity, 100 d milk yield, 100 d milking frequency and retained foetal membrane) were shortlisted after univariable survival analyses ($P < 0.25$) and were tested by multivariable survival analyses. Year and parity were significant ($P < 0.05$) in the final model.

In the final model, 100 d milking frequency was not associated with the interval from calving to first oestrus event, however, cows with milking frequencies greater or equal to 2.3 times/day had a greater likelihood of being observed in oestrus by 49 days in milk than cows with lower milking frequencies (Table 3). The relative likelihood of recording first oestrus event by 49 days in milk decreased gradually with the year of automatic milking systems operation, with cows in year 5 being less (HR: 0.32; $P < 0.001$) likely to be observed in oestrus by 49 days in milk compared to cows in year 1. In addition, primiparous cows had a lower rate of coming in oestrus than their multiparous herd mates. When the final model was fitted after excluding 100 d milking frequency, no significant association was observed between 100 d milk yield and the interval from calving to first oestrus. The final model was also fitted after excluding 100 d milk yield but no significant association was observed between 100-days milking frequency and the interval from calving to first oestrus.

Interval from calving to first insemination

First insemination event was recorded for all the lactations examined in this study. Out of the 15 predictor variables (listed in Table 1) tested, ten (year, season of calving, parity, season of first insemination, 100 d milking frequency, 100 d protein percent, breed, retained foetal membrane, 100 d milk yield and assisted calving) were shortlisted after univariable linear regression analyses ($P < 0.25$) and were further tested by multivariable linear regression analyses. Season of calving and season of first insemination presented a significant ($P < 0.05$) effect on interval from calving to first insemination; however, no significant effect ($P = 0.09$) of their interaction was noted.

No significant association was observed between time to first insemination and milking frequency or milk yield during the first 100 days of lactation (Table 4). Interval from calving to first oestrus was significantly ($P < 0.001$) associated with the interval from calving to first insemination (Table 4). Cows that calved during autumn had the shortest (69 d) average interval from calving to first insemination (Table 4). The final model was fitted after excluding 100 d milking frequency, but no significant association ($P = 0.84$) was observed between 100 d milk yield and the interval from calving to first insemination. The final model was also fitted after excluding 100 d milk yield, however, no significant association was observed in cows between 100 d milking frequency and the interval from calving to first insemination.

Interval from calving to conception

Among the 798 lactations investigated, cows had a positive pregnancy diagnosis for 86% ($n = 686$) lactations. Out of the 15 predictor variables (Table 1) tested by univariable analysis, seven (twin, assisted calving, interval from calving to first oestrus, calving season, retained foetal membrane, milk fever and 100 d milk protein percent) were shortlisted after univariable analyses ($P < 0.25$) and were further tested by multivariable survival analyses. Interval from calving to first oestrus and season of calving were significant ($P < 0.05$) in the final model.

No significant association was observed between time to conception and milking frequency or milk yield during the first 100 days of lactation (Table 5). Season of calving played a significant role in influencing the likelihood for conception. Conceptions occurred at a 43% greater rate in autumn than in summer, at any point in time ($P < 0.05$). For each additional day between calving and first oestrus, the rate of conception reduced by about 1%. The final model was refitted after including all the significant predictors and excluding 100 d milking frequency. No significant association ($P = 0.71$) was noted between 100 d milk yield and the interval from calving to conception. The final model was also fitted after excluding 100 d milk yield, however, no significant association was observed.

Number of inseminations per conception

Among the 798 lactations investigated, for 686 (86%) lactations cows had a positive pregnancy diagnosis and the dataset of those cows was used for analysis of number of inseminations per conception.

Out of the 15 predictor variables (Table 1) initially tested by Poisson regression, seven (season of conception, twin, year, 100 d milk yield, retained foetal membrane, parity and milk fever) were shortlisted after univariable analyses ($P < 0.25$) and were further tested by multivariable analysis. Season of conception and twin birth ($P < 0.05$) but not their interaction had a significant effect ($P = 0.60$) on the number of inseminations per conception.

Milking frequency or milk yield during 100 days in milk were not significantly associated with number of inseminations per conception (Table 6). Cows that were inseminated during autumn months required significantly less ($P < 0.05$) inseminations per conception compared with cows that were inseminated during other seasons. Cows that gave birth to twins required more ($P < 0.05$) inseminations per conception compared to cows that gave birth to a singleton (Table 6). No significant association was noted between 100 d milk yield and number of inseminations per conception after excluding 100 d milking frequency prior to fitting the final model. The final model was also fitted after excluding 100 d milk yield, however, no significant association was observed in cows between 100 d milking frequency and the number of inseminations per conception.

Probability of submission for insemination by 80 days in milk

Initially predictor variables (listed in Table 1, except the interval from calving to first oestrus) were tested in univariable analyses and of these eight variables (season of calving, year, parity, season of insemination, 100 d milk yield, assisted calving, 100 d milking frequency and breed) significant at $P < 0.25$ were tested by multivariable survival analyses. Two variables (season of calving and season of first insemination) were significant ($P < 0.05$) but the interaction of these two variables was not significant ($P = 0.99$).

Neither 100 d milk yield nor milking frequency was significantly associated with the probability of submission for insemination by 80 days in milk (Table 7). Cows that calved and were inseminated during autumn were more likely (odds ratio: 5.59 and 1.81 respectively) to be inseminated by 80 days in milk compared with those calved or inseminated in summer (Table 7). No significant association ($P = 0.49$) was observed between 100 d milk yield and probability of submission for insemination by 80 days in milk. The final model was also fitted after excluding 100 d milk yield, however, no significant association ($P = 0.33$) was observed.

Probability of conception by 100 days in milk

Out of the 15 predictor variables (Table 1, except the interval from calving to first oestrus) tested by univariable analyses, eight (year, season of calving, season of conception, season of insemination, parity, twin, retained foetal membrane and milk fever) shortlisted after univariable logistic regression analyses ($P < 0.25$) and were tested by multivariable analysis. Year and season of calving had a significant ($P < 0.05$) effect on the probability of conception by 100 days in milk. Year and season of calving were tested for the interactions but no significant ($P < 0.22$) interaction was observed.

No significant association was observed between the probability of conception by 100 days in milk and milking frequency or milk yield during 100 days in milk (Table 8). Probability of conception by 100 days in milk decreased gradually with the time of automatic milking systems operation ($P < 0.05$). Compared with the summer calving season, calving in autumn increased (odds ratio = 2.15) the likelihood of a cow to be in calf by 100 days in milk (Table 8). The final model was fitted after excluding 100 d milking frequency; however, no significant association was noted between 100 d milk yield and conception by 100 days in milk. The final model was also fitted after excluding 100 d milk yield, however, no significant association was observed in cows in different quartiles of 100 d milking frequency and conception by 100 days in milk.

Neither milk yield nor milking frequency from calving to 100 days in milk was significantly associated with the probability of conception at first insemination. The outcome of the univariable analysis indicated that year of automatic milking systems commissioning, parity and retained foetal membrane were significant at $P < 0.25$, however no significant association of these variables was noted with the probability of conception at first insemination.

Discussion

Descriptive statistics

The average intervals from calving to first insemination and calving to conception in our study (87 and 128 d, respectively) are longer than the findings of other Australian researchers who reported the corresponding intervals of 76 and 101 d (Stevenson and Lean, 1998); 83 and 118 d (Moss et al., 2002) respectively. In the current study, submission for insemination by 80 days in milk and conception rate by 100 days in milk (36% and 49%, respectively) were lower than the industry benchmarks (58% and 73%, respectively; InCalf, 2007). Morton (2004) analysed the data of Australian dairy herds during 1996 and 1997 and reported 66% submission rate for insemination by 80 days in milk and 53% pregnancy rate by 100 days in milk. However, the declining trend of reproductive performance during last ten years (Morton, 2011) supports and could, at least in part, explain the relative reduction of the reproductive outcomes in the current study.

Milking frequency

No significant influence of 100 d milking frequency on reproductive measures were observed in this study, however, the probability of first oestrus to be recorded by 49 days in milk ($P = 0.13$) and conception by 100 days in milk ($P = 0.18$) tended to be highest for cows milked at least 2.3x/d averaged compared to cows milked ≤1.5x/d. The association between milking frequency during early lactation and reproductive outcome is a matter of debate. In a conventional milking system, McNamara et al. (2008) observed no association between milking frequency and reproductive performance. Increased milking frequency in automatic milking system did neither cause a delay in resumption in ovarian cyclicity nor related to an increase in interval from calving to conception (Weiss et al., 2004). However, Patton et al. (2006) observed shorter interval from calving to first oestrus for cows milked once a day compared with milking three times per day. Similarly Blevins et al. (2006) reported an increase in days from calving to first oestrus in cows milked four times a day compared with cows milked twice a day. It has been reported that cows milking themselves in automatic milking systems (regardless of the milking frequency) had an increase in the interval from calving to first insemination compared to the cows milked in conventional milking systems (Kruip et al., 2002). The low incidence of very high milking frequencies in our study may explain the lack of significant effect of milking frequency on reproductive performance, indeed, only 1% of cows averaged three or more milkings/day during the first 100 days in milk. The fact that higher milking frequencies tended to be associated with increased likelihood of first oestrus could be explained by the concept that these cows moved around the system voluntarily more frequently and may have gained access to more fresh allocations of feed, thereby reducing the duration of early lactation negative energy balance. It is also possible that cows with higher milking frequencies were inadvertently observed by farm staff more frequently, which could have increased the chance of an oestrus event being detected.

Milk volume and milk solids

One of the primary objectives of the study was to determine the association between reproductive performance and milk yield. Milk yield was not significantly associated with any of the reproductive measures evaluated here. Whilst managed in a conventional milking system, using the same herd for cows lactating from February 2005 until January 2006, Pedernera et al. (2008) also reported the non-significant relationship between milk yield and reproductive performance. This finding is consistent with other studies indicating the neutral effect of milk production on the interval from calving to conception in United State Holstein cows (Gröhn and Rajala-Schultz, 2000) and on sub-fertility in Australian dairy herds (Moss et al., 2002).

Discrepancies exist in literature to explain the effect of milk yield on fertility. Previous studies have reported negative (Mann et al., 2005; Ranasinghe et al., 2010) and positive (López-Gatius et al., 2006) effects of milk production on fertility. However, the relationships between milk yield and reproductive performance varies according to the herd level as well as on individual animal level (Löf et al., 2007). For example, Windig et al. (2005) reported that herds with high average milk yield had shorter calving to first AI intervals but within herds, cows with high production had longer calving to first AI intervals. Moreover, a number of factors such as negative energy balance, hormonal concentrations in blood, fertility of sire, accuracy of oestrus detection and insemination technique are interrelated for fertility (Gröhn and Rajala-Schultz, 2000; Lucy, 2001) and obviously healthy cows producing more milk are inseminated sooner compared with less healthy cows (Gröhn and Rajala-Schultz, 2000). Thus, conception and first insemination are potentially influenced more by managerial decisions and individual animal disease history rather than milk production level *per se* (Eicker et al., 1996). Moreover, the association between milk yield and conception is difficult to interpret due to the confounding effect of culling (Gröhn and Rajala-Schultz, 2000). Cows that do not conceive with repeated inseminations and have low milk yield are more prone to be culled (Gröhn and Rajala-Schultz, 2000).

Whilst there was no consistent trend in the number of inseminations per conception for the different milk yield groups, cows with a 100 day milk yield in the range of 1950 to 2521 kg had the lowest number of inseminations per conception ($P = 0.06$) whilst cows in the next production group (2522 to 3044 kg milk) had the highest number of inseminations per conception. One possible explanation for these differences could be the slightly higher (31 versus 27%) incidence of diagnosed mastitis before conception in the cows producing milk in the range of 2522 to 3044 kg compared with cows producing milk from 1950 to 2521 kg during 100 days in milk.

Previous studies (Fahey et al., 2003; Haile-Mariam, 2003) have shown positive effects of milk protein on reproductive performance. Although 100 d milk protein percentage was associated with the interval from calving to first oestrus by the univariable analyses (data not shown), the final model indicated that neither milk protein nor fat percentage was significantly associated with any of the tested reproductive measures.

Time relative to automatic milking systems commissioning

The probability of conception by 100 days in milk decreased gradually with the time of automatic milking systems commissioning. Though the likelihood of first oestrus decreased steadily in relation to year of automatic milking systems commissioning, the general lack of reported long term reproductive performance data with automatic milking systems makes it challenging to interpret this result. It is possible that this is a farm specific finding that could be explained by reduced culling on reproductive performance as the herd size was gradually increased and/or an increased acceptance of low fertility cows as the herd progressively moved away from split calving to year round calving. Although the herd was managed as a predominantly split calving herd early in the study period, but there were no months recorded when at least one cow was not inseminated. Certainly, without the inclusion of data from additional farms there is no ability to conclude that automatic milking systems directly caused the trend. It is also possible that the disruption to the herd and farm staff routines associated with the installation and testing of the RR during year 3 to year 5 influenced the effectiveness of oestrus detection during these years.

Year of automatic milking systems commissioning was not associated with any changes in either interval from calving to first insemination or the likelihood of conception in the current study. However, an earlier study (Dearing et al., 2004) has reported increased intervals from calving to conception and decreased conception rate during year one compared to the year prior to an automatic milking systems being commissioned under research conditions. On the other hand, Löf et al. (2007) found that herds with automatic milking systems had shorter duration of calving to first insemination and conception compared with herds milked conventionally.

Season of calving

Days to first insemination were significantly shorter in autumn calved cows compared with cows calved in other seasons which is in agreement with data published by Hammoud et al. (2010). These authors explained the positive influence of autumn calving on reproductive performance by having optimal environments and availability of fresh green pastures through the early lactation period (Hammoud et al., 2010). Although cows are not typically classified as seasonal breeders, changes in photoperiodic stimulation (Dahl et al., 2000) associated with specific times of the year could be a potential explanation for such an effect of season on the reproductive measures (Santos et al., 2009). Alternatively, increased levels of pasture allocation and improved health condition explain (at least in part) such findings. Data relating to pasture allocation and daily herd feeding levels were not incorporated into this analysis.

Twin birth

Association of twin births with an increase in the number of inseminations required per conception in the current study is consistent with other reports (Nielen, 1989; Bicalho et al., 2007). These authors reported that cows giving birth to twins were less (odds ratio: 0.78) likely to conceive and 1.42 times more likely to be culled compared with dams with singleton births.

Parity

In our study, significant relationship between calving to first oestrus and parity was observed. Numerous studies have reported negative (Darwash et al., 1997; Gröhn and Rajala-Schultz, 2000; Moss et al., 2002), positive (Alawneh et al., 2011) or neutral (Horan et al., 2005) effect of parity on reproductive outcomes in conventional milking system. Poorer reproductive performance in primiparous cows than multiparous cows can be explained by having low pre-calving live weight in heifers (Morton, 2004) that may cause increased postpartum anovulatory interval amongst Friesian heifers (Burke et al., 1995). In the present study, parity was included in each final model to avoid any confounding effects. It is possible that the effect reported in the present study was impacted by the nutritional status of the cows and it is possible that the regularity of access to fresh feed was highest for the fourth parity cows and gradually reduced for both younger and older cows.

Interval from calving to first oestrus

The time to first insemination was influenced by time to first oestrus that is in agreement with findings of Galvão et al. (2010) and Dubuc et al. (2012) who reported that cows that were cycling by 49 days in milk had an increased median time to first insemination compared to cows that started cycling after 49 days in milk. Abnormal resumption of postpartum ovarian cycles, reduced oestrus detection efficiency and decreased oestrus expression are all common causes for extended interval from calving to insemination (Muhammad et al., 2011). Washburn et al. (2002) reported an increase in interval from calving to first insemination (84 to 100 d) which was attributed to a decline in oestrus detection rate (51 to 42%).

We noted a significant relationship between increasing interval from calving to first oestrus and a reduced number of inseminations per conception on univariable analysis (data not presented). However, on multivariable analysis, the association was not significant. Previous studies have also reported the positive effect of longer days to first oestrus with probability of pregnancy (Friggens and Labouriau, 2010) and a reduction of the incidence of repeated inseminations in conventional milking system (Moss et al., 2002) in agreement with our findings. Such a relationship could be the result of positive effects of an increased number of ovulatory cycles and more cyclic pattern of reproductive hormones preceding insemination for better uterine involution and other physiological changes associated with improved implantation (Butler and Smith, 1989; Diskin et al., 2003).

We did not find any significant association ($P < 0.05$) of the periparturient disorders; retained foetal membrane, milk fever or stillborn in respect to any outcome variable in the final models despite these being reportedly potential risk factors for reduced reproductive performance in other studies (Gröhn and Rajala-Schultz, 2000; López-Gatius et al., 2006). The low incidence of these diseases in the dataset presented here is the most likely reason of the lack of significance.

Among the factors investigated, interval from calving to first oestrus, season of calving, year of automatic milking systems commissioning and twin birth were the key factors influencing reproductive performance in the automatic milking systems research herd. However, it is noted that one of the challenges of working with historical data is that often the decision-making that sits behind the management is not captured which creates some difficulties with data interpretation. It is likely that the dataset presented here was also impacted by factors which included (but would not be limited to) shifting from split calving to year-round calving gradually during the study period and the targeted increase in herd size and associated reduced voluntary culling.

Conclusion

Overall, this study has provided an insight into the factors that contributed to reproductive performance of dairy cows in a pasture-based automatic milking systems research farm. At the reported production levels and milking frequencies for Australian pastured cow, milk yield and milking frequency during 100 days in milk had no effect on reproductive measures. Interval from calving to first oestrus increased gradually within the study period and consequently influenced other reproductive outcomes. Besides this, season of calving, season of insemination, conception, twin birth all affected the reproductive performance in the automatic milking systems research herd. With these results in mind, reviewing management strategies and initiating operating procedures to minimize the identified risk factors would be warranted.

References

Alawneh JI, Laven RA, Stevenson MA (2011) The effect of lameness on the fertility of dairy cattle in a seasonally breeding pasture-based system. *Journal of Dairy Science* **94**, 5487-5493.

Bicalho RC, Cheong SH, Galvão KN, Warnick LD, Guard CL (2007) Effect of twin birth calvings on milk production, reproductive performance, and survival of lactating cows. *Journal of the American Veterinary Medical Association* **231**, 1390-1397.

Blevins CA, Shirley JE, Stevenson JS (2006) Milking frequency, estradiol cypionate, and somatotropin influence lactation and reproduction in dairy cows. *Journal of Dairy Science* **89**, 4176-4187.

Burke CR, Mcdougall S, Macmillan KL (1995) Effects of breed and calving liveweight on postpartum ovarian activity in pasture-fed dairy heifers. In ´Proceedings of the New Zealand Society of Animal Production´. pp.76-78. (New Zealand Society of Animal Production: New Zealand)

Butler WR, Smith RD (1989) Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science* **72**, 767-783.

Dahl GE, Buchanan BA, Tucker HA (2000) Photoperiodic effects on dairy cattle: A Review. *Journal of Dairy Science* **83**, 885-893.

Darwash AO, Lamming GE, Wooliams JA (1997) The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. *Animal Science* **65**, 9-16.

de Koning K (2011) Automatic Milking: Common practice on over 10,000 dairy farms worldwide. In ´Proceedings of the Dairy Research Foundation Symposium´. pp. 14-31. (The University of Sydney, Camden, NSW, Australia)

de Koning CJAM, Rodenburg J (2004) Automatic Milking: State of the art in Europe and North America. In ´Automatic milking – A better understanding´. (Ed. A Meijering, H Hogeveen, C J A M de Koning) pp. 27-40. (Wageningen Academic Publishers, Wageningen, the Netherlands)

Dearing J, Hilerton JE, Poelarends JJ, Neijenhuis F, Sampimon OC, Fossing C (2004) Effects of automatic milking on body condition score and fertility of dairy cows. In ´A better understanding automatic milking´. (Ed. A Meijering, H Hogeveen, C J A M de Koning) pp. 135-140. (Wageningen Academic Publishers, The Netherlands)

Dhand NK (2009) SAS macros for statistical modelling. Available at http://sydney.edu.au/vetscience/biostat/macros/multi_about.shtml. (verified July 04 2012)

Dhand NK (2010) UniLogistic: A SAS macro for descriptive and univariable logistic regression analyses. *Journal of Statistical Software* **35**(1), 1-15.

Diskin MG, Mackey DR, Roche JF, Sreenan JM (2003) Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle. *Animal Production Science* **78**, 345-370.

Dubuc J, Duffield TF, Leslie KE, Walton JS, Leblanc SJ (2012) Risk factors and effects of postpartum anovulation in dairy cows. *Journal of Dairy Science* **95**, 1845-1854.

Eicker SW, Gröhn YT, Hertl JA (1996) The association between cumulative milk yield, days open, and days to first breeding in new york holstein cows. *Journal of Dairy Science* **79,** 235-241.

Fahey J, Morton J, Macmillan KL (2003) Relationships between milk protein percentage and reproductive performance in Australian dairy cows. In ´Proceedings of the New Zealand Society of Animal Production´. pp. 82-86. (New Zealand Society of Animal Production: New Zealand)

Friggens NC, Labouriau R (2010) Probability of pregnancy as affected by oestrus number and days to first oestrus in dairy cows of three breeds and parities. *Animal Reproduction Science* **118**, 155-162.

Galvão KN, Frajblat M, Butler WR, Brittin SB, Guard CL, Gilbert RO (2010) Effect of early postpartum ovulation on fertility in dairy cows. *Reproduction in Domestic Animals* **45**, 207-211.

Gröhn YT, Rajala-Schultz PJ (2000) Epidemiology of reproductive performance in dairy cows. *Animal Reproduction Science* **60**, 605-614.

Haile-Mariam M, Morton JM, Goddard ME (2003) Estimates of genetic parameters for fertility traits of Australian Holstein-Friesian cattle. *Animal Science (Penicuik, Scotland)* **76**, 35-42.

Hammoud MH, El-Zarkouny SZ, Oudah EZM (2010) Effect of sire, age at first calving, season and year of calving and parity on reproductive performance of Friesian cows under semiarid conditions in Egypt. *Archiva Zootechnica* **13**, 60-82.

Horan B, Mee JF, O'connor P, Rath M, Dillon P (2005) The effect of strain of Holstein-Friesian cow and feeding system on postpartum ovarian function, animal production and conception rate to first service. *Theriogenology* **63**, 950-971.

InCalf (2007) Available at http://www.incalf.com.au. (verified September 07, 2012)

Kolbach R, Kerrisk KL, Garcia, SC (2013) Short communication: The effect of pre milking with a teat cup–like device, in a novel robotic rotary, on attachment accuracy and milk removal. *Journal of Dairy Science* **96**, 360-365.

Kruip TM, Morice H, Robert M, Ouweltjes W (2002) Robotic milking and its effect on fertility and cell counts. *Journal of Dairy Science* **85**, 2576-2581.

Löf E, Gustafsson H, Emanuelson U (2007) Associations between herd characteristics and reproductive efficiency in dairy herds. *Journal of Dairy Science* **90**, 4897-4907.

López-Gatius F, García-Ispierto I, Santolaria P, Yániz J, Nogareda C, López-Béjar M (2006) Screening for high fertility in high-producing dairy cows. *Theriogenology* **65**, 1678-1689.

Lucy MC (2001) Reproductive loss in high-producing dairy cattle: where will it end? *Journal of Dairy Science* **84**, 1277-1293.

Mann GE, Mann SJ, Blache D, Webb R (2005) Metabolic variables and plasma leptin concentrations in dairy cows exhibiting reproductive cycle abnormalities identified through milk progesterone monitoring during the post partum period. *Animal Reproduction Science* **88**, 191-202.

Mcnamara S, Murphy JJ, O'mara FP, Rath M, Mee JF (2008) Effect of milking frequency in early lactation on energy metabolism, milk production and reproductive performance of dairy cows. *Livestock Science* **117**, 70-78.

Morton J (2004) ´Determinants of reproductive performance of dairy cows in commercial herds in Australia´. PhD thesis, The University of Melbourne, Australia.

Morton J (2011) Report on InCalf Fertility Data Project, 2011. Dairy Australia, Southbank, Victoria.

Moss N, Lean IJ, Reid SWJ, Hodgson DR (2002) Risk factors for repeat-breeder syndrome in New South Wales dairy cows. *Preventive Veterinary Medicine* **54**, 91-103.

Muhammad Y, Toshihiko N, Chikako Y, Su TL, Gokarna G, Bimalka KR, Kana K, Aki H (2011) Days in milk at first AI in dairy cows; its effect on subsequent reproductive performance and some factors influencing it. *Journal of Reproduction and Development* **57**, 643-649.

Nielen M, Schukken YH, Scholl DT, Wilbrink HJ, Brand A (1989) Twinning in dairy cattle: A study of risk factors and effects. *Theriogenology* **32**, 845-862.

Patton J, Kenny DA, Mee JF, O'mara FP, Wathes DC, Cook M, Murphy JJ (2006) Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. *Journal of Dairy Science* **89**, 1478-1487.

Pedernera M, Garcia SC, Horagadoga A, Barchia I, Fulkerson WJ (2008) Energy balance and reproduction on dairy cows fed to achieve low or high milk production on a pasture-based system. *Journal of Dairy Science* **91**, 3896-3907.

Ranasinghe RMSBK, Nakao T, Yamada K, Koike K (2010) Silent ovulation, based on walking activity and milk progesterone concentrations, in Holstein cows housed in a free-stall barn. *Theriogenology* **73**, 942-949.

Rossing W, Ipema AH, Veltman PH (1985) IMAG Research Report 85-2 the feasibility of milking in a feeding box. (Wageningen, The Netherlands)

Santos JEP, Rutigliano HM, Filho MFS (2009) Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. *Animal Reproduction Science* **110**, 207-221.

Stevenson MA, Lean IJ (1998) Descriptive epidemiological study on culling and deaths in eight dairy herds. *Australian Veterinary Journal* **76**, 482-488.

Talukder S, Celi P, Kerrisk KL, Garcia SC, Dhand NK (2012) Reproductive performance of dairy cows in a pasture based Automatic Milking System research farm: a retrospective analysis. In ´Proceedings of the fifth Australasian Dairy Science Symposium´. pp. 302-305. (Melbourne, Australia)

Washburn SP, Silvia WJ, Brown CH, Mcdaniel BT, Mcallister AJ (2002) Trends in reproductive performance in Southeastern Holstein and Jersey DHI herds. *Journal of Dairy Science* **85**, 244-251.

Weiss D, Reist M, Bruckmaier RM (2004) The acyclic period postpartum in automatic and conventional milking. *Journal of Veterinary Medicine Series A* **51**, 268-272.

Windig JJ, Calus MPL, Veerkamp RF (2005). Influence of herd environment on health and fertility and their relationship with milk production. *Journal of Dairy Science* **88**, 335–347.

Table 1. Descriptions and values of variables evaluated

Name	Descriptions	Values
Year	Year of automatic milking system operation in which lactations started	Year 1, Year 2, Year 3, Year 4, Year 5[A]
Season of calving	Season in which cow calved	Summer, Autumn, Winter, Spring[B]
Season of first insemination	Season in which cow was first inseminated	Summer, Autumn, Winter, Spring[B]
Season of conception	Season in which cow conceived	Summer, Autumn, Winter, Spring[B]
100 d milk yield	Cumulative milk yield (kg) during first 100 days in milk as recorded by inline milk meters	≤ 1950, 1950 to 2521, 2522 to 3044, >3044
100 d milking frequency	Average daily number of milkings through first 100 days in milk	≤1.5, 1.6 to 1.9, 2.0 to 2.2, ≥2.3
100 d milk fat percent	Milk fat percent at first 100 days in milk	≤3.28, 3.29 to 3.64, 3.65 to 4.01, >4.01
100 d milk protein percent	Milk protein percent at first 100 days in milk	≤2.88, 2.89 to 3.04, 3.05 to 3.19, >3.19
Interval from calving to first oestrus	Interval (d) from date of calving to date of first detected oestrus	Continuous
Parity	Lactation number	1, 2, 3, 4, 5, ≥6
Twin	Record of twin calving	Yes, No
Assisted calving	Record of requiring any assistance during calving	Yes, No
Retained foetal membrane	Retention of placenta for at least 24 hours post- parturition	Yes, No
Still birth	Parturition of dead calf	Yes, No
Milk fever	Record of any clinical sign of milk fever within four weeks of parturition	Yes, No

[A] Year 1 = Calving from 1st May 2006 to 30th April 2007; Year 2 = 1st May 2007 to 30th April 2008; Year 3 = 1st May 2008 to 30th April 2009; Year 4 = 1st May 2009 to 30th April 2010; Year 5 = 1st May 2010 to 30th April 2011.

[B] Summer = December to February; Autumn = March to May; Winter = June to August; Spring = September to November.

Table 2. Descriptive statistics of continuous production and outcome variables for 798 lactations from 365 cows in a pasture-based automatic milking system research herd from May 2006 to April 2011

Variables	Mean	SD	Median	Q1	Q3
100 day milk yield, kg	2491	706.6	2523	1959	3049
100 d milking frequency	1.9	0.5	1.9	1.6	2.2
100 d milk fat percent	3.7	0.6	3.7	3.3	4.0
100 d milk protein percent	3.0	0.3	3.0	2.9	3.2
Estimated 305-day milk yield, kg	6093	1706	6200	4922	7281
Interval from calving to first oestrus, d	-	-	57	40	175
Interval from calving to first insemination, d	87	33	79	61	200
Interval from calving to conception, d	-	-	112	76	298
Number of inseminations per conception	1.9	1.2	1.0	1	6

s.d: standard deviation: Q1: Lower quartile; Q3: Upper quartile

In 38% (*n = 305*) of lactations, cows were recorded for at least one oestrus event by 49 days in milk and their data were used for the analyses of interval from calving to first oestrus. In 86% (*n = 686*) of lactations, cows had a positive pregnancy diagnosis and were used for the analyses of the interval from calving to conception and number of inseminations per conception. For other variables, 798 lactations were used for descriptive statistics.

Table 3. Factors affecting the interval from calving to first oestrus in dairy cows in a pasture-based automatic milking system research herd[A] based on Cox proportional regression model analyses

Predictor variables	Class	HR	95% CI[B]	*P*-value
Year	Year 1	1.00*	-	<0.001
	Year 2	1.28	0.9-1.8	
	Year 3	0.87	0.6-1.3	
	Year 4	0.55	0.4-0.8	
	Year 5	0.32	0.2-0.5	
Parity	1	1.00*	-	0.045
	2	1.24	0.8-2.0	
	3	1.62	1.0-2.6	
	4	1.40	0.8-2.4	
	5	2.21	1.3-3.7	
	≥6	1.22	0.7-2.0	
100 d milking frequency	≤1.5	1.00*	-	0.12
	1.6 to 1.9	1.56	1.1-2.3	
	2.0 to 2.2	1.43	0.9-2.2	
	≥2.3	1.62	1.0-2.5	
100 d milk yield (kg)	≤ 1950	1.00*	-	0.80
	1950 to 2521	0.86	0.6-1.3	
	2522 to 3044	0.92	0.6-1.5	
	>3044	0.81	0.5-1.3	

Results are presented as hazard ratio (HR), confidence interval (CI) and probability values.

Hazard ratio (HR) is the ratio of the hazard rates corresponding to the event (e.g., oestrus) described by different levels of an explanatory variable. For example, if a cow has a hazard ratio of 1.28 (year 2), then she has a 28 % increased likelihood of recorded oestrus compared with a cow in year 1.

[A] Covariates in the final model included season of calving.

[B] 95% CI for HR.

* Reference category.

Table 4. Factors affecting the interval (days) from calving to first insemination in dairy cows in a pasture-based automatic milking system research herd[A] based on linear mixed model analyses

Step[B]	Predictor variables	Class	n	Ratios of GM (95% CI)	LSM	SEM	95% CI[C]	*P*-value
1	Interval from calving to first oestrus, d	Continuous	-	-	0.0063 *	0.0004* *	0.006-0.007	<0.001
2	Season of calving	Summer	150	-	88.1[a]	3.0	82.3-94.2	<0.001
		Autumn	169	0.8 (0.7-0.8)	68.7[b]	2.1	64.7-73.1	
		Spring	101	1.0 (0.9-1.1)	83.1[a]	3.0	77.4-89.3	
		Winter	132	1.0 (0.9-1.1)	83.4[a]	3.0	77.7-89.5	
3	Season of insemination	Summer	90	-	76.3[b]	3.0	70.6-82.4	0.001
		Autumn	207	1.1 (1.0-1.2)	80.7[b]	2.3	76.3-85.3	
		Spring	129	1.0 (0.9-1.1)	74.9[b]	2.8	69.6-80.6	
		Winter	126	1.2 (1.1-1.4)	91.1[a]	3.1	85.2-97.4	
4	100 d milking frequency	≤1.5	136	-	80.6	2.3	76.3-85.1	0.60
		1.6 to 1.9	159	1.0 (0.9-1.1)	80.6	1.9	75.9-85.4	
		2.0 to 2.2	139	1.0 (1.0-1.1)	82.5	2.2	78.3-86.9	
		≥2.3	118	1.0 (0.9-1.1)	79.4	2.5	74.6-84.4	
5	100 d milk yield (kg)	≤ 1950	147	-	79.2	2.5	74.4-84.4	0.80
		1950 to 2521	139	1.0 (0.9-1.1)	79.8	2.2	75.7-84.2	
		2522 to 3044	133	1.0 (1.0-1.1)	80.7	2.1	76.6-84.9	
		>3044	138	1.1 (1.0-1.2)	82.3	2.3	77.9-86.9	

Results are presented as ratio of geometric means, least squares means (LSM), standard error of mean (SEM), confidence interval (CI) and probability values.

GM denotes geometric means. Ratios of geometric means were obtained by exponentiation of the regression coefficients.

Means within a column and step with different lowercase superscript letters are significantly different (*P* ≤ 0.05).

[A] Covariates in the final model included parity.

[B] Step refers to the step at which each variable was entered in the statistical model.

[C] 95% CI for LSM.

[*] Estimate

** Standard error

Table 5. Factors affecting the interval from calving to conception in dairy cows in a pasture-based automatic milking system research herd[A] based on Cox proportional regression model analyses

Predictor variables	Class	HR	95% CI[B]	*P*-value
Interval from calving to first oestrus, days	Continuous	0.99	0.991-0.996	<0.001
Season of calving	Summer	1.00*	-	0.003
	Autumn	1.43	1.2-1.8	
	Spring	1.11	0.9-1.4	
	Winter	1.00	0.8-1.3	
100 d milking frequency	≤1.5	1.00*	-	0.64
	1.6 to 1.9	0.95	0.8-1.2	
	2.0 to 2.2	0.85	0.6-1.1	
	≥2.3	0.91	0.7-1.2	
100 d milk yield (kg)	≤ 1950	1.00*	-	0.65
	1950 to 2521	1.19	0.9-1.6	
	2522 to 3044	1.13	0.8-1.6	
	>3044	1.16	0.8-1.7	

Results are presented as hazard ratio (HR), confidence interval (CI) and probability values.

Hazard ratio (HR) is the ratio of the hazard rates corresponding to the event (e.g., conception) described by different levels of an explanatory variable. For example, if a cow has a hazard ratio of 1.43 (calving season: autumn), then she had a 43% increased likelihood of conception compared with a cow calved in summer.

[A] Covariates in the final model included parity.

[B] 95% CI for HR.

* Reference category.

Table 6. Factors affecting the number of inseminations per conception in dairy cows in a pasture-based automatic milking system research herd[A] based on Poisson regression analyses

Step[B]	Predictor variables	Class	n	Ratios of counts (95% CI)	LSM	SEM	95% CI[C]	*P*-value
1	Season of conception	Summer	86	-	2.5	0.3	2.0-3.0	0.006
		Autumn	187	0.8 (0.6-0.9)	1.9	0.2	1.6-2.2	
		Spring	149	0.9 (0.8-1.1)	2.3	0.2	1.9-2.7	
		Winter	211	1.0 (0.8-1.2)	2.4	0.2	2.0-2.8	
2	Twin	Yes	22	1.4 (1.0-1.8)	2.6	0.3	2.0-3.4	0.028
		No	611		1.9	0.1	1.8-2.0	
3	100 d milk yield (kg)	≤ 1950	154		2.2	0.2	1.8-2.6	0.059
		1950 to 2521	160	1.0 (0.8-1.1)	2.1	0.2	1.7-2.5	
		2522 to 3044	163	1.2 (1.0-1.4)	2.5	0.2	2.1-3.0	
		>3044	156	1.0 (0.8-1.3)	2.2	0.2	1.8-2.6	
4	100 d milking frequency	≤1.5	144	-	2.2	0.2	1.8-2.6	0.70
		1.6 to 1.9	185	1.1 (0.9-1.3)	2.3	0.2	2.0-2.7	
		2.0 to 2.2	162	1.0 (0.9-1.3)	2.3	0.2	1.9-2.7	
		≥2.3	142	1.0 (0.8-1.2)	2.1	0.2	1.7-2.6	

Results are presented as the ratio of counts, least squares means (LSM), standard error of mean (SEM), confidence interval (CI) and probability values.

Ratios were obtained by exponentiation of the regression coefficients.

Means within a column and step with different lowercase superscripts significantly different ($P \leq 0.05$).

[A] Covariates in the final model included parity, season of calving.

[B] Step refers to the step at which each variable was entered in the statistical model.

[C] 95% CI for LSM.

Table 7. Factors affecting submission by 80 days in milk in dairy cows in a pasture-based automatic milking system research herd[A] based on logistic mixed model analyses

Step[B]	Predictor variables	Class	n	OR	95% CI[C]	*P*-value
1	Season of calving	Summer	171	1.00*	-	<0.001
		Autumn	198	5.59	3.1-10.1	
		Spring	114	1.75	0.9-3.6	
		Winter	151	2.48	1.1-5.4	
2	Season of first insemination	Summer	97	1.00*	-	0.001
		Autumn	240	1.81	0.9-3.8	
		Spring	160	0.93	0.5-1.9	
		Winter	137	0.45	0.2-1.0	
		No	609	2.98	1.1-8.0	
3	100 d milk yield (kg)	≤ 1950	155	1.00*	-	0.44
		1950 to 2521	160	0.84	0.5-1.5	
		2522 to 3044	163	1.16	0.6-2.1	
		>3044	156	0.79	0.4-1.6	
4	100 d milking frequency	≤1.5	144	1.00*	-	0.33
		1.6 to 1.9	185	1.46	0.9-2.5	
		2.0 to 2.2	162	0.99	0.6-1.8	
		≥2.3	143	1.25	0.7-2.4	

Results are presented as the odds ratio (OR), confidence interval (CI) and probability values.

[A] Covariates in the final model included parity.

[B] Step refers to the step at which each variable was entered in the statistical model.

[C] 95% CI for odds ratio.

* Reference category.

Table 8. Factors affecting conception by100 days in milk in dairy cows in a pasture-based automatic milking system research herd[A] based on logistic mixed model analyses

Step[B]	Predictor variables	Class	*n*	OR	95% CI[C]	*P*-value
1	Year	Year 1	117	1.00*	-	0.001
		Year 2	122	0.72	0.4-1.3	
		Year 3	109	0.63	0.4-1.1	
		Year 4	131	0.33	0.2-0.6	
		Year 5	155	0.41	0.2-0.7	
2	Season of calving	Summer	171	1.00*	-	0.005
		Autumn	198	2.15	1.4-3.4	
		Spring	114	1.05	1.6-1.8	
		Winter	151	1.40	0.8-2.3	
3	100 d milking frequency	≤1.5	144	1.00*	-	0.18
		1.6 to 1.9	185	1.15	0.7-1.9	
		2.0 to 2.2	162	0.92	0.5-1.6	
		≥2.3	143	1.61	0.8-3.0	
4	100 d milk yield (kg)	≤ 1950	155	1.00*	-	0.67
		1950 to 2521	160	0.84	0.5-1.4	
		2522 to 3044	163	0.73	0.4-1.3	
		>3044	156	0.67	0.3-1.3	

Results are presented as the odds ratio (OR), confidence interval and probability values.

[A] Covariates in the final model included parity.

[B] Step refers to the step at which each variable was entered in the statistical model.

[C] 95% CI for odds ratio.

* Reference category.