Egypt. J. Vet. Sci. **Vol. 56**, No. 5, pp. 863-874 (2025)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Cow, Season and Disease Risk Factors Associated with Subfertility in Holstein Cows

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Abstract

IN THIS study, we examined the association of some risk factors with subfertility in Holstein cows under Egypt's subtropical climate. Lactations (n = 1832) from a high-yielding (305-d milk yield ≈ 10,000 kg) herd were enrolled. Only 10% of cows became pregnant to first AI, and 21.2% were pregnant by 120 days in milk, (PREG120). The likelihood of PREG120 decreased significantly in high-yielding cows, after spring and summer calvings, and when the first AI occurred after 65 days in milk (DIM). For cows with lameness, mastitis, or reproductive problems prior to pregnancy, the odds of PREG120 decreased by 38%, 68%, and 36%, respectively. Increased milk production (odds ratio, OR: 1.54 for moderate, and 2.21 for high producers), summer calving (OR: 2.18), and affection with lameness (OR: 1.71), mastitis (OR: 2.57), or reproductive disorders (OR: 1.29) increased the risk of repeat breeding compared to low-producing, winter calving, and non-affected cow, respectively. Unadjusted mean number of services per conception was 4.29. Cows in the moderate- and high-milk groups, cows who calved in seasons other than fall, and those affected with lameness or mastitis all required more AI for pregnancy. In conclusion, fertility of cows in the herd under investigation was suboptimal. To sustain fertility in subtropical conditions, cows should be shielded during hot months, bred earlier than 65 DIM, and cows suffering from dystocia, retained placenta, metritis, lameness, or mastitis should be properly handled to minimize their detrimental impact on subsequent fertility. Also, breeding methods should be tailored for high-producing animals with special consideration paid to their condition.

Keywords: Dairy, cow, subfertility, risk, factors.

Introduction

Declining fertility is a well-recognized global issue that affects the dairy business [1, 2]. Subfertility is a major cause of economic losses and is a major limitation to the achievement of optimum production efficiency in dairy cattle enterprises [3]. Thus, optimal reproductive efficiency is a priority for sustainability and for guaranteeing profitability in dairy cows.

A range of impacts, including genetic, environmental, and managerial factors, influence fertility in high-milk-producing dairy cows [4]. Despite some studies [5] have associated a decline in herd fertility with the rise in milk production, others [6] have criticized this link and indicated that there are number of other factors underlying the relationship. Parity effects on fertility were extensively studied; however, results were inconsistent among studies with greater evidence that primiparous cows are more fertile than pluriparous [7-11]. Cow's fertility is vulnerable to climatic effects.

Heat-stressed cows show reduced fertility in terms of longer intervals to conception, lower conception rates and higher pregnancy losses [12, 13]. Some studies reported a link between stage after calving at first breeding and subsequent fertility. For example, Stangaferro et al. [14] found increased risk of culling multiparous cows given a voluntary waiting period of 88 d compared to 60 d. Also, the hazard of pregnancy by 210 DIM was 85% less in

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(Received 30/03/2024, accepted 20/05/2024)

DOI: 10.21608/EJVS.2024.252415.1977

cows with long (≥80 d vs. <80 d) calving to first insemination interval [11].

Health problems, especially those that arise early in lactation, are frequent in dairy cows. Carvalho et al. [15] found that 30% of cows developed a clinical disease within 21 DIM, and [16] determined that 61% of cows were diagnosed with at least one postpartum disorder within 60 DIM, which had carryover effects on dairy cow reproduction, milk production, and culling. Through bacterial endotoxins and inflammatory mediators, intramammary infection has a deleterious impact on ovarian function, estrus expression, and uterine environment, which are important for healthy fertility [17, 18].

Similarly, negative effects on fertility have been reported due to lameness. It was associated with a decrease in estrus behavior [19], a lower chance of cyclicity and pregnancy, and longer times from calving to first insemination and to conception [20]. Disorders related to the reproductive system e.g. dystocia, retained placenta, and metritis complex, either individually or grouped, hinder cow fertility, and are among the principle reasons of culling cows and direct economic losses [16, 21, 22]. A calving interval of 12 to 13 mo is widely accepted as an economically effective target to maximize lifetime production [23]. Cows conceiving prior to 120 DIM [24] with 1.5 - 1.7 services per conception [25] would achieve the target.

Repeat breeding, defined as a failure to conceive after three or more inseminations in the absence of abnormalities, is reflection of increased NSC and extended service period, and is a common sign of subfertility [26, 27]. Identification of factors that impede the success of conception could be useful in managing reproduction and enhancing profitability of dairy cows. In instances where reproductive performance is regarded acceptable, several studies have undertaken the risk of infertility e.g. [27, 28], but little is known about the risk factors when the fertility level is poor. Therefore, the purpose of the present study was to investigate some cow, environment and disease risk factors potentially associating subfertility in a high yielding (average 305-d milk yield ≈ 10,000 kg) low fertile herd of Holstein cows in Egypt's subtropical climate.

Material and Methods

This study was approved by the Research Ethics Committee, and the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Alexandria University, Egypt (approval 49 number 202199).

Study Herd

Data of the current investigation are lactation and health records of Holstein cows raised in a large commercial dairy herd located approximately 82 km on Cairo-Alexandria desert road, Egypt. Files were retrieved from the management software (DairyComp305, Valley Ag Software, Tulare, CA).

Cows were kept under intensive management system with zero grazing, and calving and milking are year-round. They were housed free in fenced yards with shades and dirt floor. Lactating cows were grouped according to level of milk production, and the concentrate feeding was offered accordingly. They were fed a total mixed ration (TMR) twice daily throughout the year.

The TMR consisted of concentrates, corn silage, wheat bran, alfalfa hay, vitamins, minerals, and calcium bicarbonate. When available, green berseem or its hay, and rice or wheat straw was fed. Water is available at all times. Heifers are artificially inseminated for the first time when reaching about 360 kg of body weight. Non-pregnant cows were inspected visually for estrus twice daily by herd personnel. Insemination was based on the AM-PM rule using imported frozen Holstein semen.

Planned voluntary waiting period is 50 to 60 days after calving, and pregnancy diagnosis is done by rectal palpation on day 42 post-insemination. Cows were machine milked three times a day at eight-hour intervals starting at 06:00 a.m., and daily milk yield was recorded electronically for individual cows. Cows were dried off about 60 d prior to the anticipated calving date.

Data Handling and Statistical Analysis

The initial data file contained individual performance records from 2040 cows calving between years 2016 and 2017 without repetition per cow. Records with missing basic information e.g., pregnancy status, and those outside the following intervals were excluded from the analysis: 10 to 220 days in milk (DIM) at first breeding (DIMFB), and 20 to 400 days open. After editing, the maximum number used in descriptive statistics and hypothesis testing was 1832 (89.8%) lactations. The reproductive variables considered were pregnancy by 120 DIM (PREG120), repeat breeder (RB) cows and number of services per conception (NSC).

Repeat breeders are cows that failed to conceive after three or more inseminations within the same lactation [22]. We coded the variables PREG120 and RB as binary outcomes (0, 1), where 0 referred to a cow that did not become pregnant by 120 DIM (for PREG120) or a cow that required less than four AI to become pregnant during her open period (for RB).

Number of services per conception represents the number of AI a cow received throughout her calving to conception. We analyzed all data with SPSS for Windows (version 22, IBM Corp., Armonk, NY, USA). To describe the proportion of pregnant cows during the first 120 DIM, Kaplan-Meier survival curve was generated. Non-pregnant cows by 120 DIM and cows died or culled for reasons other than low fertility during the specified period were censored. Frequency graphs were created to illustrate the distribution of DIMFB and number of AI a cow received between calving and conception or calving to last insemination up to 400 days open.

The association of the success variables (PREG120 and RB) with the possible risk factors was assessed using multivariable binary logistic regression. Parameter estimates including odds ratio and its 95% confidence interval were presented. Proportion of PREG120 and RB cows was analyzed by the generalized linear models (GZLM) with a binomial distribution and a logit link function.

Number of services per conception was analyzed by GZLM with Poisson distribution and a log link function. Least squares means and standard errors of means from GZLM were reported, and in all analyses, marginal means were compared after Bonferroni adjustment. We considered a probability (P) value less than or equals to 0.05 statistically significant. The independent variables potentially associating the risk of PREG120 and RB or affecting NSC were the same in the three analyses. They were categorized as follows: parity (first, second and third or higher), realized or expected current lactation 305-d milk yield (low, < 9,100 kg; moderate, 9,100 to 11,000 kg and high > 11,000 kg based on the approximate lower (< mean – 0.5 SD), middle (mean ± 0.5 SD) and upper (> mean + 0.5 SD) thirds of the distribution, calving year (2016 and 2017), calving season (winter, December to February; spring, March to May; summer, June to August and fall, September to November), and DIMFB (below average, ≤ 65 d and above average, > 65 d).

Disease risk factors included lameness, reproductive disorders and mastitis. We coded a disease (1 = no, 2 = yes) as happened (= yes) when the date of its occurrence was earlier than that of the target reproductive outcome. A cow was recorded as ‘yes’ for reproductive disorders if she was diagnosed and treated for one or more of the following conditions, difficult calving, placental retention, and/or vaginal/uterine and infections. Locomotion scoring is accomplished by observing cows while standing and walking with emphasis on their back posture for early detection of lameness. All diseases were diagnosed and treated by farm resident veterinarians. All main effects and significant (P <0.05) biologically relevant two-way interactions were retained in the final models. Calving year was included in all models to remove potential confounding, but estimates not presented.

Results

In the present study, a total of 2040 lactation records from cows in a large commercial dairy herd were examined; 208 (10.2%) records were excluded because of missing information and data restriction boundaries leaving 1832 lactations for analysis. The mean intervals from calving to first observed estrus, and to first AI were 43.7 and 67.8 days, respectively. On average, cows required 4.3 services and 173 DIM (median 149 d) to conceive (Table 1). Approximately half (50.5%) of the cows received their first insemination between 60 and 80 DIM, most (87.9%) of them had a first AI between 50 and 90 DIM, and practically all cows (99.1%) were bred by 130 DIM (Figure 1). The survival curve in Figure 2 showed that 13.5%, 17.5% and 21.2% of cows became pregnant by 85, 100 and 120 DIM, respectively. The distribution of times bred in Figure (3) demonstrated that only 10% of cows became pregnant to first AI. Cumulative conception rate after a second through a fifth AI was 21.9%, 32.5%, 40.5% and 46.3%, respectively.

Pregnancy by 120 Days in Milk

Parameter estimates of the multivariable binary logistic regression for the likelihood of PREG120, and the least squares means for the proportion of cows PREG120 categorized by levels of the possible risk factors are listed in Table (2). The likelihood of PREG120 decreased with increasing milk yield in the current lactation. Based on the percentage change in odds ratio, high-yielding cows were 49% (P = 0.003) less likely to be PREG120 compared to low-yielding cows, and the mean proportion of PREG120 in low-producer cows was 9% higher (P≤0.05). Season of calving was associated with the risk of PREG120. Taking calving in winter as a reference category, there was a lower odds of PREG120 for spring (OR: 0.58, P = 0.002) and summer calvings (OR: 0.50, P = 0.006). Also, lower proportions (P<0.05) of cows calving in spring and summer months were PREG120 compared to calvings in winter and fall months. Inseminating cows for the first time after 65 DIM was negatively associated with the odds of PREG120 (OR: 0.60, P<0.001), and the proportion of cows becoming PREG120 was 7% less (P<0.01) than that when first AI occurred at earlier DIM. The predicted odds of PREG120 were decreasing by 38% (P = 0.007), 68% (P < 0.001), and 36% (P<0.01) for cows affected with lameness, mastitis, and reproductive disorders, respectively. The corresponding proportion of cows PREG120 was 6, 15 and 6 percentage points lower compared to non-affected ones.

Repeat Breeder Cows

Least squares means and standard errors for the proportion of RB cows, as well as parameter estimates of the multivariable binary logistic regression for the association of RB with the possible risk factors are presented in Figure (4). Factors significantly associated with RB were level of milk production in the current lactation, season of calving, and cows suffering lameness, mastitis or reproductive disorders. Moderate (OR: 1.54, P = 0.031) and high (OR: 2.21, P < 0.001) milk yield in the current lactation, summer calving (OR: 2.18, P < 0.001), and affection with lameness (OR: 1.71, P = 0.001), mastitis (OR: 2.57, P < 0.001), or reproductive disorders (OR: 1.29, P = 0.044) were all associated with increased risk of RB compared to low-producing, winter calving, and non-affected cow, respectively. Cows initiating their lactations in fall months 50% ($P < 0.001$) were less likely, and those that calved during summer months were 2.18 ($P \leq 0.001$) times more likely to be RB compared to winter calvings. Also, significantly ($P < 0.05$ or $P < 0.01$) higher mean proportions of RB were observed in high vs. moderate and low milk-producer cows, summer calving vs. other seasons, and in cows positive for lameness, mastitis and reproductive disorders.

Number of Services per Conception

Unadjusted mean and standard deviation of NSC was 4.29 and 2.71 (Table 1). Table (3) showed that cows in the moderate- and high-milk groups required 0.77 and 1.65 ($P<0.01$) more AI for pregnancy than low-producer cows. Cows that calved during the winter, spring and summer months required 0.48, ($P<0.05$), 0.60 ($P<0.01$) and 1.22 ($P<0.01$), respectively more AI to become pregnant compared to fall calvings. The NSC was higher in cows suffering lameness (+0.44, $P<0.01$) and mastitis (+1.05, $P<0.01$) than non-affected cows.

Discussion

In the current study, the interval between calving and first detected estrus, as well as the interval between calving and first breeding, are not excessively long. Other fertility measures, on the other hand, indicate that fertility of cows in the herd under consideration is suboptimal. Because only 10% of cows were pregnant to first AI, a low conception rate was a major contributor in the herd's poor reproduction. On average, cows required more than four AI to conceive, and only one fifth of cows were pregnant by 120 DIM. This efficiency is outlying that described in the literature for dairy cows [3, 27, 29]. We looked at whether the characteristics linked to poor fertility elsewhere apply to high-producing (average 305-d milk yield ≈ 10,000 kg), low fertile herds in our subtropical climate.

Parity had no effect on the likelihood of PREG120, RB, or variation in NSC in the current investigation. This is in line with the findings of [7, 30], who found no significant effect of parity on NSC. Also, parity was not related to the odds of pregnancy to first service in reports of Tillard et al. [31] and Kim and Jeong [11]. However, studies signifying parity effects on fertility have been controversial. Impaired reproduction and a higher number of inseminations were associated with lower parity [8], although, in other studies higher parity number was associated with lower odds of conception [9, 10, 32], increased NSC [29], and higher likelihood of RB [22].

We found a negative relationship between level of milk yield and the three fertility parameters. Cows with high and moderate milk output were less likely to become PREG120, were more likely to be RB, and were inseminated more frequently for pregnancy than cows with low milk yield. Energy shortage during early postpartum due to increased energy output in milk is hypothesized to mediate the deteriorating effect of high milk yield on fertility [33]. Negative energy balance and altered hypothalamic-pituitary-ovarian activity are accompanied by metabolic alterations, and inhibition of ovarian follicle and corpus luteum growth and development [1, 34]. The subject of whether intensity of milk production is linked to reproductive efficiency is still being debated among scientists. Our results are in agreement with some previous reports, but contradict others. For example, Siatka et al. [5] found that high-milking cows required 0.6 more services to conceive than low-milking group. One risk factor for being a RB, according to [35], was milk production. The risk rose 1.5 times with a daily milk yield rise of approximately 15 kg fat-corrected milk. On the other hand, [9] found that increased early (4th week) milk output was connected with increased odds of a cow becoming pregnant by 100 DIM and 150 DIM. López-Gatius et al. [28] found that high-producer cows had a higher likelihood (OR: 6.8) of high fertility (pregnant before 90 DIM), and that each 1 kg decrease in milk yield during peak production was associated with a 1.8-day increase in the calving to conception interval. According to Rearte et al. [10], despite the statistical evidence of a negative association between milk yield and fertility, the magnitude of the relationship was so minor that its biological impact is essentially non-existent. The use of different indices of production and fertility, the bias resulting from varied management and culling decisions, as well as different data handling approaches, may be the reasons for such disparities among research results [6, 36].

According to the findings of the present study, calving season significantly associated the odds of PREG120 and RB, and affected NSC in favor of calvings occurring in the cooler winter and fall seasons. Because their comfort zone (temperature-humidity index, THI < 72, De Rensis et al., [37]) is frequently exceeded during the warmer months of spring and summer in Egypt (meteorological data in El-Tarabany and El-Tarabany, 2015b [13]), lactating cows are prone to heat stress in these seasons. Our study, therefore, supports the hypothesis that cows starting their lactations under heat stress may subsequently suffer inferior reproductive performance than those calving in more favorable climate. Previous research on Holsteins raised in Egypt's subtropical conditions demonstrated also that hot seasons have a detrimental impact on cow fertility. When the THI was high (80–85 points) at conception, fetal loss, abortion, and stillbirth were higher, and pregnancy per AI at 28 and 75 d post service were lower than when THI was low (< 70 points), as well, DO was delayed by 27 days [12, 13]. Similar seasonal pattern and impact were demonstrated by researchers in other countries [7, 11]. The adverse effects of heat stress on cow's fertility is multi-dimensional and are mediated through changes in feed intake and energy balance, the neuroendocrine function, estrus behavior, ovarian follicle development and oocyte quality, ability of fertilization, and reduced embryonic survival [37, 38].

When compared to earlier inseminations, breeding later than 65 days post-calving resulted in a 7% reduction in the proportion of pregnant cows, and a 40% reduction in the likelihood of becoming pregnant by 120 DIM. With a shorter time between calving and initial AI cows may have more opportunity for earlier and more frequent insemination. In agreement, cows with delayed first AI had prolonged calving to conception interval [14] and lower first service conception rate [11, 32]. In other investigations, however, with a short calving to first AI interval, conception to first service was lower [31, 39], and the proportion of RB cows increased [35].

The results of the present study demonstrated that lameness was associated with smaller odds and a lower proportion of cows PREG120, an increased proportion and risk of RB, and more services per pregnancy relative to non-lame cows. The negative relationship between lameness and cow fertility is widely agreed in the literature. In agreement to ours, Buch et al. [40] reported that lame cows needed more services per conception. Similarly, [27] found that cows experiencing infectious interdigital disease or claw horn lesions during the service period were 30% more likely to remain open by 120 DIM, and 90% more likely to become a RB compared to unaffected cows. In addition, Omontese et al. [20] found that the pregnancy rate by 150 DIM was 12% lower, and the hazard of pregnancy was 16% lower in Jerseys with foot lesions compared to healthy cows. Beyond the evident pain, lameness has behavioral and physiological consequences, such as longer lying time, decreased locomotor activity, shorter eating time, more negative energy balance and reduced body condition [41, 42]. Lame cows have a decreased ability to demonstrate estrus behavior because they spend less time standing, restricting their ability to show sexual behavior [19]. Lameness was associated with delayed ovarian activity in Holstein cows [43], a lower ability to ovulate [44], a higher incidence of ovarian cysts [45], and a reduced odds of cyclicity [20].

In this study, clinical mastitis was consistently a highly significant risk of infertility. Reduced likelihood of becoming PREG120 and the increased risk of RB in affected cows had reflected detrimentally on the percentage of early pregnant and repeat breeding cows, as well as on NSC. Similarly, [46] found that affected cows had a decreased (OR: 0.56) chance of being pregnant by 110 DIM, and fewer (36.72%) cows got pregnant before 110 DIM than non-affected cows (50.73%). Clinical mastitis diagnosed 14 d before to 28 d after AI lowered conception rates [47]. Other researchers [30, 48] also found increased NSC in cows with various kinds of mastitis at various times post-calving. Close to our findings, Mellado et al. [27] found that cows with clinical mastitis shortly before or after the first insemination were 2.2 times more likely to require more than three services per pregnancy, and 1.4 times more likely to be non-pregnant by 120 DIM than cows with healthy udders. Gustafsson and Emanuelson [35] reported a positive relationship between the proportion of RB and the herd incidence of mastitis. Based on a review by Kumar et al. [18] low fertility in mastitis-affected cows could be the result of altered hormonal profile, impaired oocyte development or function, lower ability to ovulate, fertilisation failure, and an unfavorable uterine environment for embryonic survival and development. Bacterial toxins, which trigger the release of prostaglandin and other inflammatory mediators, are thought to underlie the negative effects of mastitis on fertility.

In this investigation, reproductive problems (dystocia, retained placenta, and/or vaginal/uterine and infections.) were regarded as a single condition (yes/no), as they had been handled in previous research that grouped health illnesses [11, 16, 26]. Results showed that reproductive disorders prior to confirmed pregnancy were significantly linked with the risk and proportions of PREG120 and RB. Because reproductive disorders are usually observed and managed around calving and early in lactation, the NSC was presumably unaffected. These results are supported by previous reports. Reproductive pathologies, e.g. dystocia, metritis, retained placenta either collectively [11] or individually [39] reduced the odds of first service conception by 45% and 46% to 79%, respectively. As pointed out by [35], the risk of RB increased in cows treated for a reproductive disease before AI (OR: 1.29). In addition, Bonneville-Hébert et al. [22] showed that a cow's chance of becoming a RB increased if she had a peripartum reproductive problem (OR: 1.13 for retained placenta, OR: 1.22 for metritis, OR: 1.44 for dystocia). Similar to ours, cows with metritis or retained placenta were 1.6 times more likely to require more than three inseminations per verified pregnancy, and those with metritis were 2.1 times more likely to remain open by 120 DIM [27]. Negative energy balance, disproportional energy metabolism, altered mineral utilization, and impaired immunological function are challenges that transition cows encounter [49]. Several cases of dystocia (29%) are followed by retained placenta and metritis [22], possibly due to peripartum immune insufficiency [50] and bacterial contamination introduced during intervention [51]. Dystocia, RP, and metritis all cause uterine involution to be delayed, as well as affecting ovarian function and cyclicity [52, 53]. All of these problems could add up to a lower fertility rate.

Conclusion

Our findings (10% pregnancy to first AI, 21.2% total pregnancies by 120 DIM, 4.29 NSC) clearly show that there was a reduced reproductive performance in the herd under investigation. Reduced fertility was linked to increased milk yield, calving in warmer months, late first postpartum AI and affection with lameness, clinical mastitis, or reproductive disorders (dystocia, RP, or metritis). To preserve their fertility under Egyptian subtropical conditions, Holstein cows should also be protected during hot months and bred sooner than 65 DIM. Cows suffering from dystocia, retained placenta, metritis, lameness, or mastitis should be adequately addressed to avoid their adverse impact on later fertility.

Conflict of Interest: The authors state that publishing this paper does not involve any conflicts of interest.

Acknowledgement: not applicable

Funding statement: Self-funding.

TABLE 1. Descriptive statistics of cow characteristics in the final dataset

Variable	Mean	SD	Median	Q1	Q3	Minimum	Maximum
Parity	2.45	1.44	2	1	3	1	9
DIMFH	43.7	21.6	42	29	57	3	200
DIMFB	67.8	18.4	65	59	76	10	220
Days open, all cows	184	88.0	167	121	238	20	400
Days open, pregnant cows	173	93.5	149	91	243	20	400
Times bred, all cows	4.00	2.80	3	2	6	1	13
Times bred, pregnant cows	4.29	2.71	4	2	6	1	13
305-d MY	10064	1916	10195	9000	11250	3030	16070

SD, standard deviation; Q1, first quartile; Q2, second quartile; DIMFH, days in milk first observed heat; DIMFB, days in milk first breeding

TABLE 2. Proportion of cows pregnant by 120 days in milk (DIM) and estimates of the multivariable binary logistic regression for the likelihood of pregnancy by 120 DIM in relation to potential risk factors

Factor	Proportion, LSM (SE)^{a}	ß	SE for ß	P value	OR	95% CI^{b} for OR
Parity						
1	0.16 (0.02)				Reference	
2	0.16 (0.02)	0.023	0.171	0.891	1.02	0.73 to 1.43
$\geq$ 3	0.17 (0.02)	0.079	0.166	0.637	1.08	0.78 to 1.50
305-d MY^{c}						
Low	0.21^{a} (0.03)				Reference	
Moderate	0.15^{ab} (0.02)	-0.399	0.208	0.056	0.67	0.45 to 1.01
High	0.12^{b} (0.02)	-0.681	0.229	0.003	0.51	0.32 to 0.79
Season						
Winter	0.21^{a} (0.03)				Reference	
Spring	0.13^{b} (0.02)	-0.548	0.174	0.002	0.58	0.41 to 0.81
Summer	0.12^{b} (0.02)	-0.686	0.252	0.006	0.50	0.31 to 0.83
Fall	0.20^{a} (0.02)	-0.010	0.182	0.956	0.99	0.69 to 1.42
DIMFB^{d}						
$\leq$ 65 d	0.20^{x} (0.02)				Reference	
> 65 d	0.13^{y} (0.02)	-0.510	0.130	<0.001	0.60	0.47 to 0.77
Lameness						
No	0.19^{x} (0.02)				Reference	
Yes	0.13^{y} (0.02)	-0.480	0.178	0.007	0.62	0.44 to 0.88
Mastitis						
No	0.25^{x} (0.02)				Reference	
Yes	0.10^{y} (0.02)	-1.154	0.173	<0.001	0.32	0.23 to 0.44
Reproductive disorders						
No	0.19^{x} (0.02)				Reference	
Yes	0.13^{y} (0.02)	-0.443	0.143	0.002	0.64	0.49 to 0.85

[a] Least squares means and standard errors for the proportion of cows pregnant by 120 DIM. Means are derived from the generalized linear models analysis with a binomial probability distribution and a logit link function.

[b] Confidence interval.

[c] 305-d milk yield of the current lactation.

[d] DIMFB, days in milk first bred.

$^{a,\, b}$ Means in a column in the same factor without a common superscript differ ($P<0.05$). $^{x,\, y}$ Means in a column in the same factor without a common superscript differ ($P<0.01$). Pairwise comparisons involving more than two marginal means were Bonferroni corrected.

TABLE 3. Number of services per conception in Holstein cows in relation to potential risk factors

Factor	LSM (SE)[a]
Parity	
1	3.97 (0.16)
2	4.00 (0.15)
≥ 3	4.23 (0.13)
305-d MY[b]	
Low	3.31^{z} (0.19)
Moderate	4.08^{y} (0.11)
High	4.96^{x} (0.15)
Season	
Winter	3.99^{bxy} (0.15)
Spring	4.11^{bx} (0.15)
Summer	4.73^{ax} (0.21)
Fall	3.51^{cy} (0.13)
DIMFB[c]	
≤ 65 d	3.97 (0.12)
> 65 d	4.15 (0.12)
Lameness	
No	3.85^{y} (0.10)
Yes	4.29^{x} (0.16)
Mastitis	
No	3.57^{y} (0.10)
Yes	4.62^{x} (0.16)
Reproductive disorders	
No	3.96 (0.13)
Yes	4.16 (0.13)

[a] Least squares means and standard errors for the number of services per pregnancy in Holstein cows. LSM (SE) are derived from the generalized linear models analysis with a Poisson probability distribution and a log link function.

[b] 305-d milk yield of the current lactation.

[c] DIMFB, days in milk first bred.

[a,b] Means in a column in the same factor without a common superscript differ (P<0.05).

[x,y] Means in a column in the same factor without a common superscript differ (P<0.01).

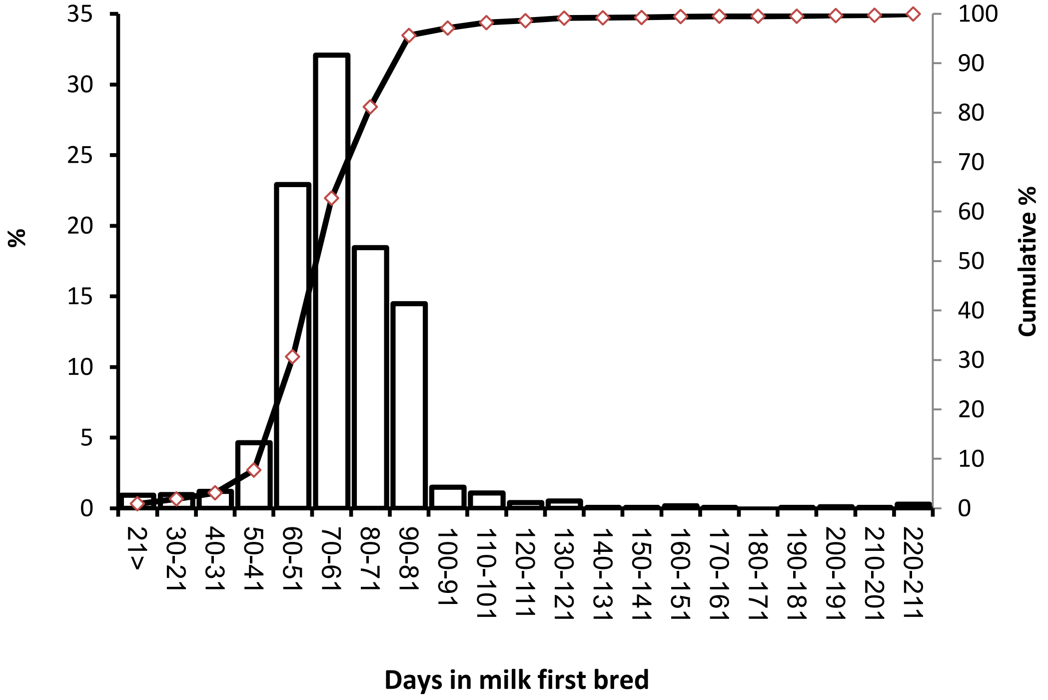

Fig.1. Distribution of cows by days in milk at first insemination. Columns are percentages (left vertical axis) of cows bred for the first time within a corresponding interval of DIM. Markers on the solid line are the cumulative percentages (right vertical axis) of cows bred for the first time by the end of the corresponding DIM.

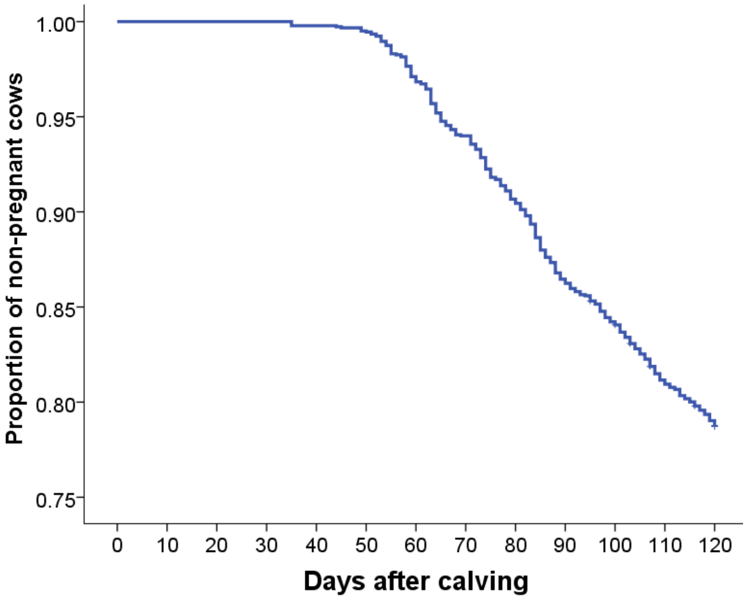

Fig.2. Kaplan-Meier survival curve showing the proportion of non-pregnant cows during the first 120 days in milk [n = 1832, censored = 1443 (78.8%), uncensored = 389 (21.2%).]

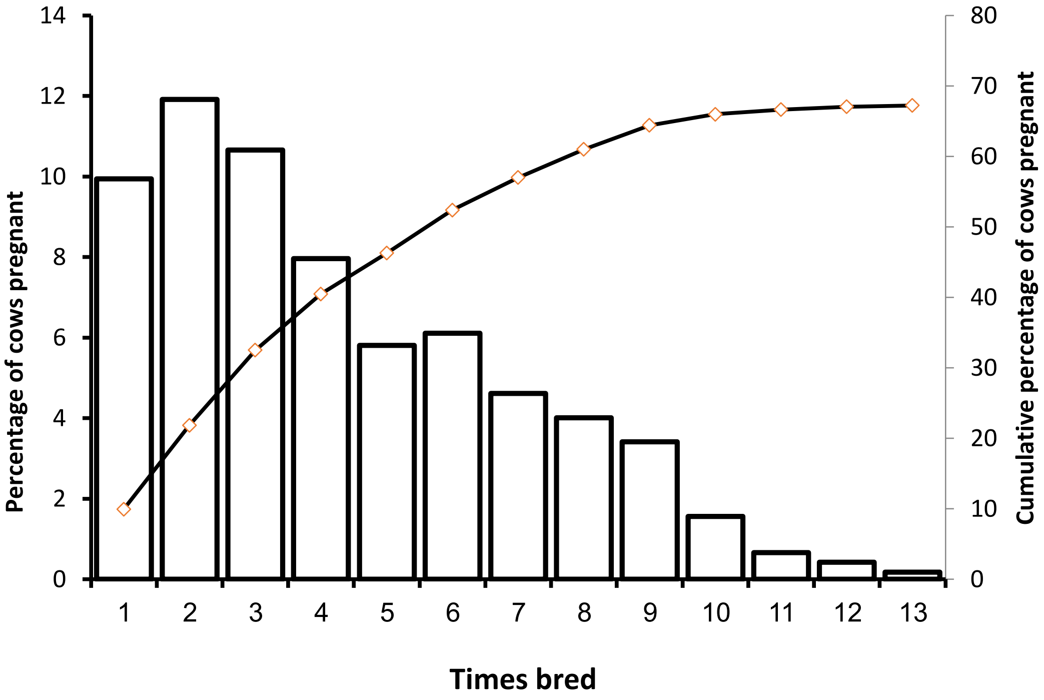

Fig. 3. Distribution of cows by number of AI they received. Columns are percentages (left vertical axis) of pregnant cows after a corresponding number of AI. Markers on the solid line are the cumulative percentages (right vertical axis) of pregnant cows.

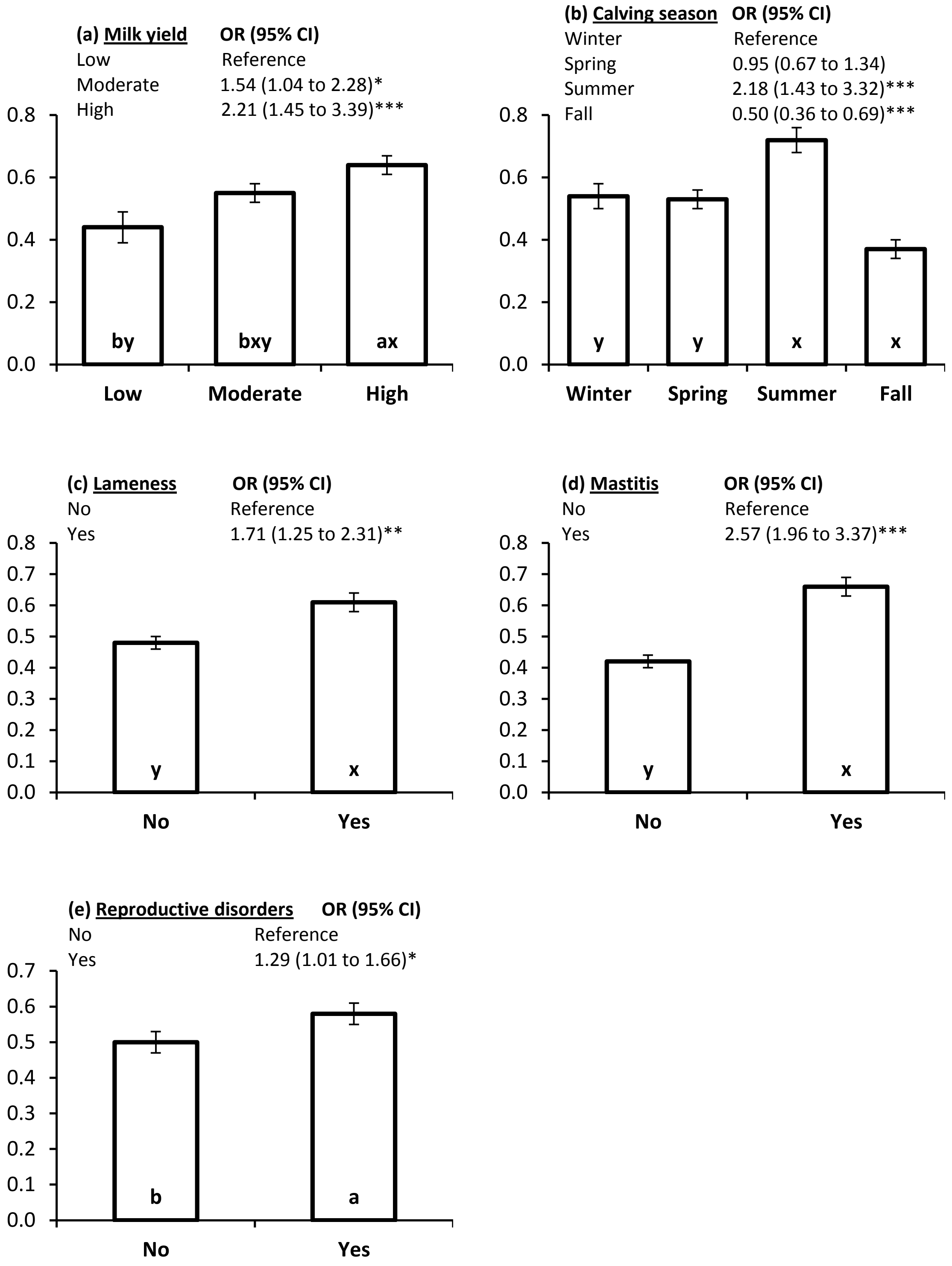

Fig.4. Proportion of repeat breeders (RB) cows and parameter estimates of the multivariable binary logistic regression for the likelihood of RB in relation to the significant risk factors. RBs are cows that fail to become pregnant after three inseminations within the same lactation. Columns with vertical bars represent least squares means and standard errors (LSM, SE) for the proportion of RB cows. LSM and SE are derived from the generalized linear models analysis with a binomial probability distribution and a logit link function. Parity and days in milk first bred (DIMFB) were not associated with RB (P>0.05). Mean (SE) proportion of RB did not differ (P>0.05) across parties; 0.52 (0.04) to 0.55 (0.03), or DIMFB ≤ 65 and > 65; 0.51 (0.03) and 0.57 (0.03), respectively. OR, odds ratio; CI, Confidence interval for odds ratio; * P<0.05, ** P<0.01, *** P<0.001. Milk yield is 305-d milk yield of the current lactation. ^{a,b} Means in the same factor without a common superscript differ (P<0.05). ^{x,y} Columns in the same factor without a common superscript differ (P<0.01). Pairwise comparisons involving more than two marginal means were Bonferroni corrected.

References

1. Lucy, M.C. Reproductive loss in high-producing dairy cattle: where will it end?. *Journal of Dairy Science*, **84**, 1277–93 (2001).
2. CDCB. Trend in daughter preg rate for Holstein or Red & White. https://queries.uscdcb.com/eval/summary/trend.cfm?R_Menu=HO.d#StartBody. Accessed August 23, (2021).
3. Inchaisri, C., Jorritsma, R., Vos, P.L., van der Weijden, G.C. and Hogeveen, H. Economic consequences of reproductive performance in dairy cattle. *Theriogenology*, **74**(5), 835-846 (2010).
4. Walsh, S.W., Williams, E.J. and Evans, A.C. A review of the causes of poor fertility in high milk producing dairy cows. *Animal Reproduction Science*, **123**(3-4), 127-38 (2011).
5. Siatka, K., Sawa, A., Czopek, S.K., Piwczyński, D. and Bogucki, M. Effect of some factors on number of services per conception in dairy cows. *Journal of Veterinary Science & Technology*, **8**, 465 (2017).
6. LeBlanc, S. Assessing the association of the level of milk production with reproductive performance in dairy cattle. *The Journal of Reproduction and Development*, **56** (Suppl, S1),7 (2010).
7. Piccardi, M., Funes, A.C., Balzarini, M. and Bo, G.A. Some factors affecting the number of days open in Argentinean dairy herds. *Theriogenology*, **79**, 760–765 (2013).
8. Lim, H.J., Yoon, H.B., Im, H., Park, J., Cho, Y.I., Jeong, Y.S., Ki, K.S. and Im, S.K. Survey on the Incidence of Reproductive Disorders in Dairy Cattle. *Journal of Embryo Transfer*, **30**, 59-64 (2015).
9. Cook, J.G. and Green, M.J. Use of early lactation milk recording data to predict the calving to conception interval in dairy herds. *Journal of Dairy Science*, **99**(6), 4699-706 (2016).
10. Rearte, R., LeBlanc, S.J., Corva, S.G., de la Sota, R.L., Lacau-Mengido, I.M. and Giuliodori, M.J. Effect of milk production on reproductive performance in dairy herds. *Journal of Dairy Science*, **101**(8), 7575-7584 (2018).
11. Kim, I.H. and Jeong, J.K. Risk factors limiting first service conception rate in dairy cows and their economic impact. *Asian-Australasian Journal of Animal Sciences*, **32**(4), 519-26 (2019).
12. El-Tarabany, M.S. and El-Tarabany, A.A. Impact of thermal stress on the efficiency of ovulation synchronization protocols in Holstein cows. *Journal of Animal Reproduction Science*, **160**,138-145 (2015a).
13. El-Tarabany, M.S. and El-Tarabany, A.A. Impact of maternal heat stress at insemination on the subsequent reproductive performance of Holstein, Brown Swiss, and their crosses. *Theriogenology*, **84**(9), 1523-1529 (2015b).
14. Stangaferro, M.L., Wijma, R., Masello, M., Thomas, M.J. and Giordano, J.O. Economic performance of lactating dairy cows submitted for first service timed artificial insemination after a voluntary waiting period of 60 or 88 days. *Journal of Dairy Science*, **101**(8), 7500-7516 (2018).
15. Carvalho, M.R., Peñagaricano, F., Santos, J.E.P., DeVries, T.J., McBride, B.W. and Ribeiro, E.S. Long-term effects of postpartum clinical disease on milk production, reproduction, and culling of dairy cows. *Journal of Dairy Science*, **102**(12), 11701-11717 (2019).
16. Macmillan, K., Gobikrushanth, M., Behrouzi, A., Hoff, B. and Colazo, M.G. Prevalence of early postpartum health disorders in Holstein cows and associations with production, reproduction, and survival outcomes on Alberta dairy farms. *Canadian Veterinary Journal*, **62**(3), 273-280 (2021).
17. Roth, Z. and Wolfenson, D. Comparing the effects of heat stress and mastitis on ovarian function in lactating cows: basic and applied aspects. *Domestic Animal Endocrinology*, **56** (Suppl, S2),18-27 (2016).
18. Kumar, N., Manimaran, A., Kumaresan, A., Jeyakumar, S., Sreela, L., Mooventhan, P. and Sivaram, M. Mastitis effects on reproductive performance in dairy cattle: a review. *Tropical Animal Health and Production*, **49**(4), 663-673 (2017).
19. Walker, S.L., Smith, R.F., Routly, J.E., Jones, D.N., Morris, M.J. and Dobson, H. Lameness, activity time-budgets, and estrus expression in dairy cattle. *Journal of Dairy Science*, **91**(12), 4552-4559 (2008).
20. Omontese, B.O., Bellet-Elias, R., Molinero, A., Catandi, G.D., Casagrande, R., Rodriguez, Z., Bisinotto, R.S. and Cramer, G. Association between hoof lesions and fertility in lactating Jersey cows. *Journal of Dairy Science*, **103**(4), 3401-3413 (2020).
21. Bell, M.J. and Roberts, D.J. The impact of uterine infection on a dairy cow's performance. *Theriogenology*, **68**(7), 1074-1079 (2007).
22. Bonneville-Hébert, A., Bouchard, E., Tremblay, D.D. and Lefebvre, R. Effect of reproductive disorders and parity on repeat breeder status and culling of dairy cows in Quebec. *The Canadian Journal of Veterinary Research*, **75**(2), 147-151 (2011).

23. Weller, J.I. and Folman, Y. Effects of calf value and reproductive management on optimum days to first breeding. *Journal of Dairy Science*, **73**(5), 1318–1326 (1990).

24. Giordano, J.O., Fricke, P.M., Wiltbank, M.C. and Cabrera, V.E. An economic decision-making support system for selection of reproductive management programs on dairy farms. *Journal of Dairy Science,* **94**(12), 6216-6232 (2011).

25. Brett, J.A. and Meiring, R.W. Evaluating Reproductive Performance on Dairy Farms. In: Hopper, RM (ed.): Bovine Reproduction. Chapter 41, P. 373. John Wiley & Sons, Inc., UK (2015).

26. Yusuf, M., Nakao, T., Ranasinghe, R.B., Gautam, G., Long, S.T., Yoshida, C., Koike, K. and Hayashi, A. Reproductive performance of repeat breeders in dairy herds. *Theriogenology*, **73**(9), 1220-1229 (2010).

27. Mellado, M., García, J.E., Véliz Deras, F.G., de Santiago, M.A., Mellado, J., Gaytán, L.R. and Ángel-García, O. The effects of periparturient events, mastitis, lameness and ketosis on reproductive performance of Holstein cows in a hot environment. *Austral Journal of Veterinary Sciences*, **50**(1), 1-8 (2018).

28. López-Gatius, F., García-Ispierto, I., Santolaria, P., Yániz, J., Nogareda, C. and López-Béjar, M. Screening for high fertility in high-producing dairy cows. *Theriogenology*, **65**(8), 1678-1689 (2006).

29. Muller, C.J.C., Potgieter, J.P., Cloete, S.W.P. and Dzama, K. Non-genetic factors affecting fertility traits in South African Holstein cows. *South African Journal of Animal Science*, **44** (1), 54-63 (2014).

30. Ahmadzadeh, A., Frago, F., Shafii, B., Dalton, J.C., Price, W.J. and McGuire, M.A. Effect of clinical mastitis and other diseases on reproductive performance of Holstein cows. *Journal of Animal Reproduction Science*, **112**(3-4), 273-282 (2009).

31. Tillard, E., Humblot, P., Faye, B., Lecomte, P., Dohoo, I. and Bocquier, F. Postcalving factors affecting conception risk in Holstein dairy cows in tropical and sub-tropical conditions. *Theriogenology*, **69**(4), 443-457 (2008).

32. Grimard, B., Freret, S., Chevallier, A., Pinto, A., Ponsart, C. and Humblot, P. Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. *Journal of Animal Reproduction Science*, **91**(1-2), 31-44 (2006).

33. Staples, C.R. and Thatcher, W.W. Relationship between ovarian activity and energy status during the early postpartum period of high producing dairy cows. *Journal of Dairy Science*, **73**, 938–947 (1990).

34. Butler, W.R. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livestock Production Science*, **83**, 211–218 (2003).

35. Gustafsson, H. and Emanuelson, U. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. *Acta Veterinaria Scandinavica*., **43**(2), 115-125 (2002).

36. Bello, N.M., Stevenson, J.S. and Tempelman, R.J. Invited review: milk production and reproductive performance: modern interdisciplinary insights into an enduring axiom. *Journal of Dairy Science*, **95**(10), 5461-5475 (2012).

37. De Rensis, F., Lopez-Gatius, F., García-Ispierto, I., Morini, G. and Scaramuzzi, R.J. Causes of declining fertility in dairy cows during the warm season. *Theriogenology*, **91**, 145-153 (2017).

38. Roth, Z. Reproductive physiology and endocrinology responses of cows exposed to environmental heat stress - Experiences from the past and lessons for the present. *Theriogenology*, **155**, 150-156 (2020).

39. Quintela, L.A., Peña, A.I., Taboada, M.J., Alonso, G., Varela Portas, B., Díaz, C., Barrio, M., García, M.E., Becerra, J.J. and Herradón, P.G. Risk factors for low pregnancy rate in dairy cattle: *a retrospective study in the north west of Spain Archivos de Zootecnia*, **53**(201), 69-76 (2004).

40. Buch, L.H., Sørensen, A.C., Lassen, J., Berg, P., Eriksson, J.Å., Jakobsen, J.H. and Sørensen, M.K. Hygiene-related and feed-related hoof diseases show different patterns of genetic correlations to clinical mastitis and female fertility. *Journal of Dairy Science*, **94**(3), 1540-1551 (2011).

41. de Vries, M.J. and Veerkamp, R.F. Energy balance of dairy cattle in relation to milk production variables and fertility. *Journal of Dairy Science*, **83**(1), 62-69 (2000).

42. Weigele, H.C., Gygax, L., Steiner, A., Wechsler, B. and Burla, J.B. Moderate lameness leads to marked behavioral changes in dairy cows. *Journal of Dairy Science*, **101**(3), 2370-2382 (2018).

43. Garbarino, E.J., Hernandez, J.A., Shearer, J.K., Risco, C.A. and Thatcher, W.W. Effect of lameness on ovarian activity in postpartum Holstein cows. *Journal of Dairy Science,* **87**(12), 4123-4131 (2004).

44. Morris, M.J., Walker, S.L., Jones, D.N., Routly, J.E., Smith, R.F. and Dobson, H. Influence of somatic cell count, body condition and lameness on follicular growth and ovulation in dairy cows. *Theriogenology*, **71**(5), 801-806 (2009).

45. Melendez, P., Bartolome, J., Archbald, L.F and Donovan, A. The association between lameness,

ovarian cysts and fertility in lactating dairy cows. *Theriogenology*, **59**(3-4), 927-937 (2003).

46. Nava-Trujillo, H. Effect of clinical mastitis on reproductive targets achievement in cows. *Veterinaria e Zootecnia*, **26**(1), 1-10 (2019).
47. Hertl, J.A., Gröhn, Y.T., Leach, J.D., Bar, D., Bennett, G.J., González, R.N., Rauch, B.J., Welcome, F.L., Tauer, L.W. and Schukken, Y.H. Effects of clinical mastitis caused by gram-positive and gram-negative bacteria and other organisms on the probability of conception in New York State Holstein dairy cows. *Journal of Dairy Science*, **93**(4), 1551-1560 (2010).
48. Elmaghraby, M.M., El-Nahas, A.F., Fathala, M.M., Sahwan, F.M. and Tag EL-Dien, M. Incidence of Clinical Mastitis and its Influence on Reproductive Performance of Dairy Cows. *The Alexandria Journal of Veterinary Sciences*, **54**(2), 84-91 (2017).
49. Esposito, G., Irons, P.C., Webb, E.C. and Chapwanya, A. Interactions between negative energy balance, metabolic diseases, uterine health and immune response in transition dairy cows. *Journal of Animal Reproduction Science*, **144**(3-4), 60-71 (2014).
50. LeBlanc, S.J. Postpartum uterine disease and dairy herd reproductive performance: a review. *Veterinary Journal*, **176**(1), 102-114 (2008).
51. Dohmen, M.J., Joop, K., Sturk, A., Bols, P.E. and Lohuis, J.A. Relationship between intra-uterine bacterial contamination, endotoxin levels and the development of endometritis in postpartum cows with dystocia or retained placenta. *Theriogenology*, **54**(7), 1019-1032 (2000).
52. Butler, W.R. and Smith, R.D. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *Journal of Dairy Science*, **72**(3), 767-783 (1989).
53. Barrier, A.C. Effects of a difficult calving on the subsequent health and welfare of the dairy cows and calves. PhD Dissertation. University of Edinburgh (2012). Available at: https://www.era.lib.ed.ac.uk/handle/1842/6527. Accessed 5 September 2021.

البقرة والموسم وعوامل الخطر المرضية المرتبطة بالخصوبة في أبقار الهولشتاين

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قمنا بدراسة بعض عوامل الخطر المرتبطة بضعف الخصوبة في أبقار الهولشتاين في ظل المناخ شبه الاستوائي في مصر. تم استخدام سجلات لبن (عدد = 1832) من قطيع عالي الإنتاجية (305 يومًا من إنتاج الحليب ≈ 10000 كجم. 10٪ فقط من الأبقار حدث لهم اخصاب من اول تلقيحه، و21.2% حدث لهم اخصاب بعد 120 يومًا في الحليب. انخفضت فرص الحمل بعد 120 يوما من الولاده (PREG120) بشكل ملحوظ في الأبقار ذات الإنتاجية العالية، بعد ولادة الربيع والصيف، و تاخير اول تلقيحة بعد 65 DIM. بالنسبة للأبقار التي تعاني من العرج أو التهاب الضرع أو مشاكل الإنجاب قبل الحمل، انخفضت الاحتمالات المتوقعه ل PREG120 بنسبة 38% و68% و36% على التوالي. ارتبطت زيادة إنتاج الحليب (نسبة الأرجحية: 1.54 بالنسبة لانتاج اللبن المتوسط و2.21 بالنسبة لانتاج اللبن العالي)، و الولادة في الصيف (نسبة الأرجحية: 2.18)، والإصابة بالعرج (نسبة الأرجحية: 1.71)، و التهاب الضرع (نسبة الأرجحية: 2.57)، و الاضطرابات الإنجابية (نسبة الأرجحية: 1.29) بزيادة خطر تكرار التلقيح (RB) مقارنة بالأبقار منخفضة الإنتاج، والولادات الشتوية، والأبقار غير المصابة، على التوالي. كان متوسط عدد التلقيحات اللازمة للاخصاب 4.29. الأبقار في مجموعات الحليب المتوسطة والعالية، والأبقار التي ولدت في مواسم غير فصل الخريف، والأبقار المصابة بالعرج أو التهاب الضرع، جميعها تطلبت اكبر عدد من التلقيحات للاخصاب (NSC). في الختام، كانت خصوبة الأبقار في القطيع قيد الدراسة دون المستوى الأمثل. للحفاظ على الخصوبة في الظروف شبه الاستوائية، يجب حماية الأبقار خلال الأشهر الحارة، وتلقيحها قبل 65 DIM، ويجب التعامل بشكل صحيح مع الأبقار التي تعاني من عسر الولادة أو المشيمة المحتبسة أو التهاب الرحم أو العرج أو التهاب الضرع لتقليل تأثيرها الضار على الخصوبة اللاحقة. كما ينبغي أن تكون طرق التربية مصممة خصيصًا للحيوانات عالية الإنتاج مع إيلاء اهتمام خاص لحالتها.

الكلمات الدالة: ابقار الحليب، ضعف الخصوبة، عوامل الخطر.