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Can post-milking insemination increase conception rate in high-producing Holstein cows under heat stress? A retrospective study

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Abstract

Heat stress, especially in countries with hot climates, is a major cause of low fertility in high-producing dairy herds. Management strategies are needed to help producers improve the reproductive performance of their dairy animals under such conditions. The current study aims to evaluate the effects of pre- and post-milking insemination on the conception rate (CR) in dairy cows. The dataset included 1294 insemination records leading to pregnancy in 708 lactating Holstein dairy cows. The GLIMMIX model procedure of SAS based on the generalized linear mixed model methodology was used to analyse the results of insemination (success or failure) as a binomial distribution with the logit link function. Differences were observed in CRs between pre- and post-milking insemination. The pregnancy odds ratio (OR) for post-milking insemination relative to that for pre-milking one was estimated at 1.90 [1.23–2.91; 95% confidence interval (CI)]. Different levels of interaction were obtained between average daily milk production and time of insemination. In the high-producing group, the pregnancy OR for the post-milking relative to that for the pre-milking insemination was estimated at 3.53 (2.00–6.24; 95% CI). A significant interaction effect was obtained between insemination time and the temperature-humidity index. A pregnancy OR of 2.52 (1.22–4.14; 95% CI) was recorded for the cows inseminated after milking on days with higher levels of heat and humidity stress *v.* the pre-milking inseminated ones. Based on the results, post-milking insemination of high-producing cows increased CRs, especially on days with high heat and humidity stress.

Introduction

Given the established relationship between dairy herd management and reproductive performance, reproductive management decisions seem to have critical economic implications (Olynk and Wolf, 2008). Reproductive performance in dairy animals affects farm profitability directly through daily milk production per cow, number of replacements available and voluntary/involuntary culling rates. Despite advances made in genetic engineering and the global progress witnessed in high-producing dairy herd management practice, reproductive efficiency seems to have suffered a dramatic decrease since the mid-1980s (Lucy, 2001). A major component of most economic indices used in the selection of dairy cows is productive life that can only be enhanced through maximizing number of parturitions during the animal's economic lifetime. Any factor causing delays or failures in pregnancy will result in reductions in the total milk produced and the calves born, ultimately leading to an increase in involuntary replacements in the herd (Sewalem *et al.*, 2008). Fertility is a multi-factorial trait whose deterioration results from an array of genetic, environmental and managerial factors. Moreover, the complex interactions of these factors make it next to impossible to determine the exact reason underlying the deteriorating traits. However, the key factors with adverse impacts on reproductive efficiency during the productive life of dairy cows have been identified (Walsh *et al.*, 2011). Low reproductive efficiency has been found to be the main cause underlying economic losses in many dairy farms (Sewalem *et al.*, 2008). This, in turn, is the result of a myriad of factors such as the nutrition system (Garnsworthy *et al.*, 2008), herd management (Olynk and Wolf, 2008; Schefers *et al.*, 2010), milk yield level (Lucy, 2001), timing of artificial insemination (AI) (Dransfield *et al.*, 1998), accuracy of oestrus detection (Schefers *et al.*, 2010), insemination process and inseminator's skills (Lima *et al.*, 2009), genital diseases such as retained placenta (López-Gatius *et al.*, 2006), climate (De Vries and Risco, 2005) and stress (Dobson and Smith, 2000). The costs imposed on breeders due to poor reproduction in cows include increased costs of re-insemination, reduced milk yield and high costs of involuntary culling (Hou *et al.*, 2009). It is, therefore, of high economic value to improve the reproductive traits of the herd. In this regard, heat stress (HS) is likely to be a major factor underlying declining productivity and low fertility in high-producing dairy herds, especially in countries with warm weather conditions. Additionally, to the best of our knowledge, there is no study relating the timing effect of AI in pre- and post-milking on conception rate (CR). Hence, the current study was designed and implemented to explore possible management practice that could alleviate or eliminate the adverse effects of heat stress on dairy cows. For this purpose, the current study strives to test the following three hypotheses:

Hypothesis 1: Conception rates are not different with regards to timing of (pre- or post-milking) AI in Holstein dairy cows.

Hypothesis 2: The timing (pre- or post-milking) effect of AI is independent of the milk yield level.

Hypothesis 3: The interaction of artificial insemination timing (pre- or post-milking) and temperature-humidity index (THI) has no effect on CR.

Materials and methods

Cows and herd management

Data were collected from a commercial Holstein dairy farm located 20 km NE of Sari, Mazandaran Province, northern Iran, (36–42.5′N latitude and 53–10.7′E longitude at an altitude of −8 m). Characterized by a generally warm and temperate climate with an annual average temperature of 16.7 °C, Sari receives most of its precipitation as rain in the winter with sporadic rainfall in the summer to yield an annual average precipitation of 690 mm (CDCW, 2013). According to the Köppen-Geiger system (Kottek *et al*., 2006), the climate in Sari is classified as warm temperate with hot dry summers when the average temperature in the warmest month reaches above 22 °C (CSA).

The farm milked around 2500 Holstein cows three times a day (at 06:00, 14:00 and 22:00 h) in a double 40 milking parlour. The cows were fed a total mixed ration without access to pasture. Feeds consisted of about 0.45 forage (maize silage, alfalfa hay and wheat straw) and 0.55 concentrate (barley, maize meal, soybean meal, sunflower meal, canola, cottonseed, wheat bran, sugar beet pulp, supplements and di-calcium phosphate). National research council recommendations were used to balance the rations (NRC, 2001).

For the purposes of the current study, dry cows were kept in a separate group to be transferred to a ‘transition group’, depending on their body condition score and age, 20–30 days prior to parturition. An early postpartum group was established as ‘fresh cows group’ for postpartum nutrition and controls, and 21-day postpartum primiparous and multiparous lactating cows were transferred to separate groups based on their daily milk production and days in milk (DIM). All animals were tested to ensure they were free of tuberculosis and brucellosis. The vaccination programmes included foot-and-mouth disease, anthrax and blackleg vaccines, regularly administered every 4 months, every 10 months and once a year, respectively. The voluntary waiting period from calving to first AI was 42 days for this herd. Lactating cows were maintained in a facility with shade, fans, sprinklers and a concrete slatted floor. Cows were housed in free stall barns bedded with sand and cleaned by scrapers three times a day. Heat detection was accomplished by visual observation three times a day for 30 min for each group of cows.

Time of insemination was recorded relative to that of milking (pre- or post-milking). Both groups were bred with frozen semen by a technician. Almost all cows were bred in the morning, such that some of the cows were inseminated within 2 h before milking and some within 2 h after milking (depending on the oestrous detection time). Any cows with metabolic and reproductive disorders and those milked ⩾3 h before or after AI were excluded from the study.

Reproductive health management, insemination and pregnancy diagnosis

Uterine discharges of cows were monitored for odours and appearance during the first 21 days of lactation. Postpartum checks (daily control) involved treatment of the following puerperal diseases until resolved or until culling: metabolic diseases such as hypocalcaemia and ketosis (the latter diagnosed during the first or second week postpartum), retained placenta (foetal membranes retained longer than 12 h after parturition) or primary metritis (diagnosed during the first or second week postpartum). The herd was maintained on a weekly reproductive health programme, involving examination of the reproductive tract of each animal by palpation per rectum within 28–30 days postpartum to check for normal uterine involution and ovarian structures. Reproductive disorders diagnosed at this time (including incomplete uterine involution, pyometra or ovarian cysts) were treated until resolved. Involution of the uterus was defined as incomplete when the uterine horns and/or cervix were larger than those in non-pregnant cows. Detectable intrauterine fluid was interpreted as pyometra. An ovarian cyst was diagnosed when a structure 20 mm or larger in diameter in either or both ovaries persisted for at least 7 days in the absence of a palpable corpus luteum. Boluses containing oxytetracycline were always administered into the uterus for cows suffering retained placenta or primary metritis. Pregnancy diagnosis was performed 38–45 days after insemination by palpation per rectum. Pregnancy was reconfirmed after 4 months and pregnant animals were re-examined after 7 months for drying.

Data structure

In the current study, information on AI treatments i.e. pre- and post-milking was gathered after the fact. The number of parturitions from July to January and the number of services from October to April were more than those in any other months of the year. A total number of 1294 inseminations (either successful or failed) were performed in 708 cows from 22 May 2011 to 21 March 2012. All the cows were from parities of one to five. Of the 1294 inseminations, 583 were administered before milking and 711 after milking. The frequency of successful and unsuccessful inseminations at each service attempt is shown in Fig. 1.

The mean and standard deviations for service numbers in the two pre- and post-milking groups were 2 ± 1.3 and 1.6 ± 0.87, respectively. Average daily milk yield during the 30 days up to the insemination ranged between 10 and 67.5 kg, with a mean of 41 ± 9.3 kg. There were 116 missing data for this trait in the post-milking insemination group. Average 30-day milk yield observations were divided into two groups of more and less than the median as low (⩽41.0 kg) and high (>41.0 kg) milk producing cows. DIM at insemination time ranged from 42 to 305 days with the mean and standard deviation of 119 ± 63.6 days, that was ultimately introduced into the final analysis as months in milk (MIM with nine levels $\text{DIM} < 60$ as $\text{MIM} = 1$ to $270 < \text{DIM} \leqslant 305$ as $\text{MIM} = 9$). Table 1 reports the frequency of cows

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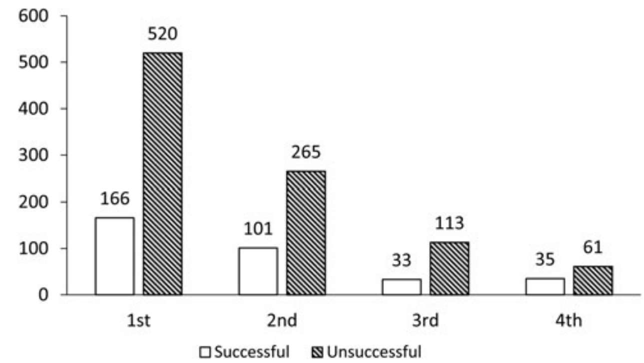

Fig. 1. Frequency distribution of successful and unsuccessful inseminations at different services.

Table 1. Frequency of services in the pre- and post-milking insemination groups and the associated milk yield levels

Service	MYL	ADIM (± SD)	AMY (kg) (± SD)
Pre-milking	⩽41 kg: 292 (0.248)	168 ± 80.1	33 ± 5.5
	>41 kg: 291 (0.247)	107 ± 52.8	48 ± 4.5
583 (0.495)	Total	137 ± 74.3	40 ± 8.6
Post-milking	⩽41 kg: 306 (0.260)	123 ± 52.7	33 ± 7.0
	> 41 kg: 289 (0.245)	90 ± 36.5	48 ± 5.4
595 (0.505)	Total	104 ± 48.1	40 ± 9.9

MYL, milk yield level: low (⩽41 kg); high (>41 kg); ADIM, average days in milk; AMY, average milk yield; SD, standard deviation

in the two milk yield groups, the pre- and post-milking insemination times and the means for DIM and milk yield.

Climate data such as daily temperature and relative humidity were taken from a meteorological station located 22 km away from the herd. The maximum THI for each day was calculated using the following equation (García-Ispierto *et al.*, 2007*a*):

$$\mathrm{THI} = (1{\cdot}8 \times T_{\max} + 32) - [(0{\cdot}55 - 0{\cdot}0055 \times \mathrm{RH}_{\min}) \times (1{\cdot}8 \times T_{\max} - 26)] \quad (1)$$

where $T_{\max}$ is the maximum temperature and $\mathrm{RH}_{\min}$ is the minimum relative humidity. For each insemination record, a THI value was assigned which was the average of the day prior to, the day of and the day after the insemination. The average THI values thus obtained were divided into two sets of below and above 70.

Statistical analysis

In the current retrospective study, all statistical analyses were conducted using the SAS package, version 9.2 (SAS Institute Inc., 2011). Descriptive statistics were determined using the UNIVARIATE and FREQ procedures. The GLIMMIX model procedure with a generalized linear mixed model methodology was used to analyse the results of insemination (success = 1 and failure = 0) as a binomial distribution with the logit link function. The model included the fixed effects of insemination time relative to milking (pre- and post-milking), parity (including the two levels of primiparous and multiparous), insemination month (six levels), MIM (nine levels), interaction effect of insemination time and parity, interaction effect of insemination time and milk yield level (less or more than the average; i.e. 41.0 kg), interaction effect of insemination time and THI levels (normal or HS conditions), and the random effect of cow. The results were presented as odds ratios (OR) and 95% confidence intervals (95% CI).

Table 2. Frequency distributions (with proportions in parentheses) of successful and failed services for cows with different insemination times within different milk yield levels

Service outcome	Time of insemination	MYL	
		Low	High
Successful	Pre-milking: 138 (0.107)	76 (0.260)	62 (0.213)
335 (0.259)	Post-milking: 197 (0.152)	59 (0.193)	101 (0.350)
Failed	Pre-milking: 445 (0.344)	216 (0.740)	229 (0.787)
959 (0.741)	Post-milking: 514 (0.397)	247 (0.807)	188 (0.650)

MYL, milk yield level: low (⩽41 kg); high (>41 kg).

Results

The frequencies of successful and failed services for cows according to insemination times (pre- or post-milking) and milk yield levels are reported in Table 2.

The results of the statistical analysis indicated that insemination time (after *v.* before milking), cow's milk yield level (high *v.* low), parity classification (primiparous *v.* multiparous), THI level (normal *v.* stressful) and their interactions with insemination time had significant effects on insemination outcome ($P < 0.05$). The comparative ORs (Table 3) indicated that cows inseminated after milking recorded a higher CR than those inseminated before milking.

The ORs of conception for post- *v.* pre-milking insemination in primiparous cows (2.6, 95% CI of 1.22–5.50), high-producing cows (3.53, 95% CI of 2.0–6.24) and cows' tolerance of stressful THI (2.24, 95% CI of 1.23–4.14) were significantly high ($P <$ 0.05), demonstrating that these cows had a greater chance of conception when inseminated after milking. Conception probability (Table 4) as predicted by the statistical model revealed noticeable differences between post- and pre-milking inseminations.

Discussion

For many years, insemination timing in cattle has been a subject of controversy over an absolute optimum time of insemination relative to ovulation, accurate determination of oestrus onset and the logistics of observing and handling animals. The current study evaluated the effects of pre- and post-milking insemination on CR in lactating Holstein dairy cows under ambient heat stress.

Table 3. ORs obtained for binary comparisons of some levels of the factors involved

Factor	First level	Second level	OR	95% CI	*T* value	Pr>|*T*|
Insemination time relative to milking	Post-milking	Pre-milking	1.90	1.23–2.91	2.93	0.004
Insemination time × Parity	Post-primiparous	Pre-primiparous	2.60	1.22–5.50	2.50	0.013
Insemination time × Parity	Post-multiparous	Pre- multiparous	1.40	1.00–2.02	1.72	0.056
Insemination time × MYL	Post-low	Pre-low	1.02	0.64–1.63	0.07	0.940
Insemination time × MYL	Post-high	Pre-high	3.53	2.00–6.24	4.36	0.001
Insemination time × THI	Post-normal[a]	Pre-normal	1.60	1.00–2.60	1.90	0.051
Insemination time × THI	Post-stress[b]	Pre-stress	2.25	1.23–4.14	2.63	0.009

OR, odds ratio; CI, confidence interval; MYL, milk yield level: low (⩽41 kg); high (>41 kg); THI, temperature-humidity index.
[a]Normal: THI < 70.
[b]Stress: THI ⩾ 70.

Table 4. Predicted CR probability under different conditions

	Post-milking insemination		Pre-milking insemination	
Condition	Probability	95% CI	Probability	95% CI
Overall	0.316 ± 0.0311	0.258–0.380	0.196 ± 0.0296	0.144–0.261
Primiparous	0.383 ± 0.0597	0.274–0.505	0.194 ± 0.0490	0.114–0.308
Multiparous	0.256 ± 0.0266	0.207–0.312	0.198 ± 0.0226	0.157–0.247
High MYL[a]	0.410 ± 0.0445	0.326–0.499	0.164 ± 0.0327	0.109–0.239
Low MYL[b]	0.235 ± 0.0316	0.179–0.303	0.232 ± 0.0353	0.170–0.309
Normal THI[c]	0.356 ± 0.0430	0.276–0.444	0.257 ± 0.0333	0.197–0.328
Stress THI[d]	0.279 ± 0.0478	0.195–0.382	0.146 ± 0.0396	0.084–0.242

CI, confidence interval.
[a]High MYL = high milk yield level: >41 kg.
[b]Low MYL = low milk yield level: ⩽41 kg.
[c]Normal THI = normal temperature-humidity index: <70.
[d]Stress THI = stress temperature-humidity index: ⩾70.

The results showed that the cows inseminated after milking recorded higher CRs than those inseminated before milking. Previous study of conditions at milking revealed elevated cortisol concentrations; increased heart-beat rates; reduced pain sensitivity, increased incidences of vocalization, kicking and stepping behaviour; as well as reduced milk yields (Van Reenen *et al.*, 2002; Wenzel *et al.*, 2003). The differences observed in CR between pre- and post-milking inseminated groups was greater among the high-producing cows.

Although little is known about the physiological factors involved in higher CR levels in post-milking inseminated cows, the oxytocin-induced stress during milking seems to be an effective parameter. Many studies have shown that oxytocin concentration rises immediately by more than 60-fold relative to its base concentration following stimulation of the teats before it declines again to its base level at the end of the milking process (Hopster *et al.*, 2002; Lollivier *et al.*, 2002; Negrao, 2008). The higher level of oxytocin receptor in the oviduct during pro-oestrus and oestrus phases induced by its higher level during milking might alter (Kotwica *et al.*, 2003) or even inhibit (Wijayagunawardane *et al.*, 2001) the contraction of different parts of the oviduct. These events may disturb local motility and secretions that are responsive to the need of the gamete transport and fertilization. However, post-milking insemination is conducted after these likely disturbances and might, hence, result in higher CRs.

The effect of HS on fertility in dairy cows is multidimensional and operates through several mechanisms that probably involve both direct physiological and reproductive effects as well as indirect metabolic and nutritional ones. Heat stress affects reproductive success in cows through its direct effects on the ovary, uterus, embryo and early foetus. These effects include diminished steroidogenesis, delayed follicle selection and a modified follicular wavelength with adverse effects on the quality of oocytes (Sartori *et al.*, 2002) as well as luteal (Bech-Sàbat *et al.*, 2008) and uterine (Thatcher *et al.*, 2001) functions. Implantation requires intricate signalling interactions between the conceptus and the mother for embryo attachment so any stress factor, including HS, can disrupt this process (Garbayo *et al.*, 2008). Previous studies reported reductions of 20–30% in CR (Morton *et al.*, 2007) and pregnancy rate (El-Tarabany and El-Bayoumi, 2015; Khan *et al.*, 2013) during the warm season. The adverse effect of high temperatures is exacerbated by high humidity, especially in high-producing dairy cows (García-Ispierto *et al.*, 2007*b*). Complex indexes, which combine some of the above climatic parameters, have been proposed to monitor the effects of HS. Thermal heat index, combining maximum temperature and

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minimum relative humidity, is the index most commonly used. The values obtained for this index indicated that higher differences in CRs were observed between the post- *v.* pre-milking inseminations during days with higher THI values (⩾70). Thus, heat stress seems to fortify the oxytocin-induced stress during milking so that pre-milking insemination leads to more stressful conditions during heat stress compared to pre-milking insemination under non-heat stress conditions. Greater difference in CRs are, therefore, not unexpected between post- and pre-milking inseminations under heat stress conditions. This difference may be partially attributed to the interaction between milk yield level and insemination time, as confirmed by the results obtained in the current study.

In summary, the current observational study showed increased CR levels during days with high THI values in post-milking inseminated cows relative to those observed in pre-milking inseminated ones, especially in high-producing cows. However, no decisive physiological evidence was detected to explain this improvement. Hence, further carefully designed physiological studies are recommended to gain a deeper insight and to take advantage of this herd management practice towards enhanced reproduction performance

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