

## Trends in Reproductive Status of Holstein Dairy Herds in Iran

### Research Article

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### ABSTRACT

This study was carried out to investigate the trends in the reproductive performance of Holstein dairy cows in Iranian commercial dairy herds. The potential effect of year, season, herd, the number of parturitions, and their interaction on reproductive performance traits were investigated on 200644 records obtained from six herds from 2007 to 2018. A nonlinearly increasing trend in calving interval (CI;  $414 \pm 1.7$  to  $418 \pm 0.74$  days) and days open (DO;  $132 \pm 1.48$  to  $136 \pm 0.58$  days) were observed. Services per conception (SPC) increased from  $1.94 \pm 0.03$  to  $2.59 \pm 0.01$  in dairy cows but altered in a fluctuating manner in heifers. The number of days from calving to first service (DFS) was decreased ( $P < 0.05$ ) from  $79 \pm 0.54$  to  $66 \pm 0.24$  days. The conception rate (CR) declined in dairy cows ( $67 \pm 0.68$  to  $58 \pm 0.31$ ;  $P < 0.05\%$ ), but no difference was observed in heifers ( $P > 0.05$ ). A decreasing trend was also recorded in age at first service (AFS) and age at the first calving (AFC) of heifers over time. Herd, season, and the number of parturitions had a significant effect on reproductive traits. Reproductive performance was higher during winter and spring compared to summer and autumn ( $P < 0.05$ ). Cows with a number of parturitions less than four had the highest reproductive performance ( $P < 0.05$ ). A similar trend is presented in fertility parameters of Holstein dairy cows showing a reduction in reproductive performance in Iranian dairy herds. Although the exact reason(s) beyond these changes are not clear, it may be due to the increment of metabolic and environmental stressors which in turn disturb the biology of reproductive system. Our results are in line with the global decline in fertility of dairy cows and emphasize on need for adopting more efficient strategies to improve reproduction status in Iranian dairy cows, especially during summer and after third parturition.

**KEY WORDS** days open, Holstein–Friesian dairy cows, number of parturitions, reproductive performance, season.

### INTRODUCTION

Herd reproductive efficiency has a great influence on profitability of dairy farms. Reproductive performance of dairy herds has decreased during recent decades in many countries (Royal *et al.* 2000; Löf *et al.* 2007; Yousefi *et al.* 2016). Several factors such as management, nutrition, and genetics practices are known to affect reproductive performance of dairy cows (Abdisa, 2018; Yousefi *et al.* 2019;

Abazarikia *et al.* 2020). Although these factors and their degree of effectiveness are dissimilar in different regions of the world (Madibela and Mahabile, 2015), management factors are common and determinant to reproductive performance of dairy cows. Several important management factors such as estrus detection method, timing of insemination, proper semen handling, and insemination, quality and fertility of semen and breeding bulls, and the skills in early pregnancy diagnosis influence dairy cows reproduction

(Foote, 2010; Reda *et al.* 2020). Moreover, genetic selection has led to a rapid increase in milk production and changed welfare of dairy cows over the years (Oltenacu and Broom, 2010). Although some reports showed no relationships between milk production at artificial insemination and conception rate (CR) (López-Gatius *et al.* 2005; García-Ispuerto *et al.* 2007), it is clear that higher milk production and negative energy balance delays resumption of ovarian activity after calving, increases days open (DO), and has a negative impact on reproductive performance in dairy cows (Melgar *et al.* 2020). For example, over 20 year, increasing annual milk yield per cow by more than 1800 kg increased DO from 112 to 152, and services per conception (SPC) from a mean of 1.9 to 2.9 (Washburn *et al.* 2002). Longer DO and the higher number of SPC, prolongs calving intervals (CI), and ultimately reduces fertility leading to increased culling rate and impose economic losses to the farm (Ahmed *et al.* 2007).

Other factors such as the number of parturitions, year, and season have also been shown to affect a herd reproductive performance. The number of parturitions of the cow significantly affected the number of SPC, DO, and CI (Fekadu *et al.* 2011). Moreover, several reproductive disorders such as repeat breeder have a great association with the number of parturitions and milk production, and increase the risk of the annual cull in dairy herds (Bonneville-Hébert *et al.* 2011).

The effects of season and year on reproductive parameters are mediated by environmental and nutritional conditions and influences age at first service (AFS) and age at the first calving (AFC) (Fekadu *et al.* 2011). In this regard, Elmetwally *et al.* (2016) showed that ovarian rebound resumes much earlier in spring. They did also report that the month of parturition affects both ovarian rebound and days open. Continuous evaluation and optimization of these risk factors are necessary for a profitable management system (Washburn *et al.* 2002).

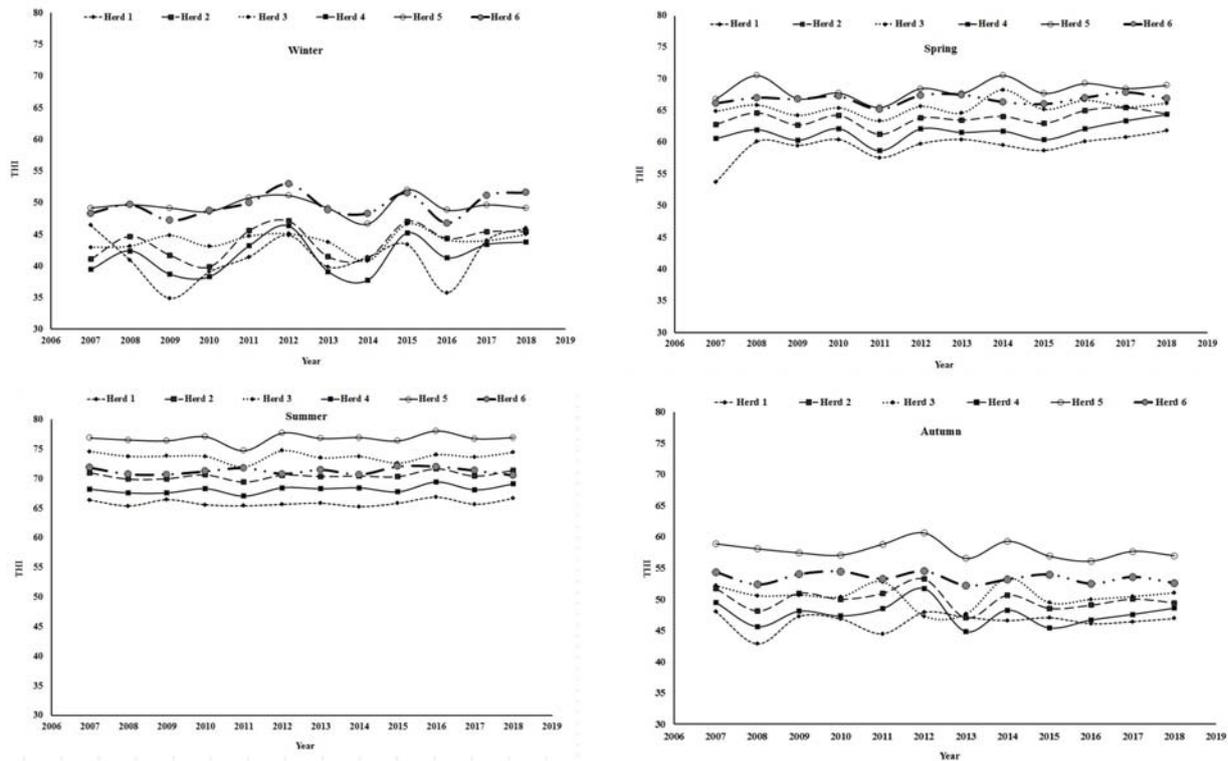
There are about eight million dairy cows in Iran, of which someone million are Holstein in more than 2000 herds. The herd sizes vary from 20 to 5000 dairy cows with mean 305-day milk production of about 8500 (range 6000 to 13700) kg (Atashi *et al.* 2012). There have been very few large-scale and long-term studies on the reproductive performance of Holstein dairy cows in Iran (Ansari-Lari *et al.* 2010; Atashi *et al.* 2012; Motlagh *et al.* 2013). More investigation on reproductive trends in Iranian commercial Holstein dairy farms, as a part of the world dairy cows' population, could help to give a deeper insight into the trend in reproductive performance and to adopt appropriate management strategies for improving reproductive outcomes in Iranian dairy herds. Our objective was to investigate the trend of changes in the fertility parameters days from calv-

ing to first service (DFS), CI, DO, CR, and SPC during the recent decade.

## MATERIALS AND METHODS

Reproductive data from six Iranian industrial dairy herds were collected from the year 2007 to 2018. All cows in different herd were fed by standard formulated diets according to the requirements as suggested by NRC (2001). These herds were under the same management systems but located in different geographical areas of Iran. The geographical coordinates of various cities are as follows: Herd 1 is located in Shahr-e Kord city at a latitude of 32° 19'32" N and longitude of 50° 51'51" E with an altitude of 2065 m above sea level; Herd 2 is located in Qazvin city at a latitude of 36° 16'07" N and longitude of 50° 00'14" E with an altitude of 1300 m above sea level; herd 3 is located in Karaj city at a latitude of 35° 49'57" N and longitude of 50° 59'29" E with an altitude of 1341 m above sea level; herd 4 is located in Khorram Darreh city at a latitude of 36° 12' 11" N and longitude of 49° 11' 13" E with an altitude of 1581 m above sea level; herd 5 is located in Sari city at a latitude of 36° 33'47" N and longitude of 53° 03'36" E with an altitude of 43 m above sea level and Herd 6 is located in Isfahan city at a latitude of 32° 39'08" N and longitude of 51° 40'28" E with an altitude of 1578 m above sea level. To evaluate possible effect of climate condition on reproduction parameters, the temperature-humidity index (THI) was calculated during different seasons of each geographical region of the dairy farm, during the experimental period (Figure 1). The seasonal THI was calculated according to the following formula (Pinto *et al.* 2020):  $THI = (0.8 \times T) + [(RH/100) \times (T - 14.4)] + 46.4$ , in which T is air temperature in °C and RH is relative humidity in percent. A presynch-Ovsynch hormonal protocol was used in all herds following a clean test. In the cases that estrus signs were detected after the second PGF2 $\alpha$  injection in Ovsynch program, artificial insemination (AI) was conducted; otherwise, the timed AI (TAI) was applied at the end of the program. Based on the time of clean test conducted on 30 days postpartum, a voluntary waiting period (VWP) of 44-50 days was considered for all the herds.

Before statistical analysis, data of cows which were out of acceptable range were omitted or adjusted according to the following criteria (Atashi *et al.* 2012): 1) AFS less than 274 or more than 639 days; 2) DFS less than 40 or more than 250 days; 3) CI less than 300 or more than 670 days; 4) the number of DO less than 50 or more than 300 days; 5) the number of inseminations more than 10 was considered as 10; 6) milk yield less than 1500 and more than 12000 during a 305-day milk yield period, and 7) dry period less than 10 or more than 300 days.



**Figure 1** Seasonal temperature-humidity index (THI) in Holstein dairy farms during the experimental period (from 2007 to 2018)

The edited dataset included summaries from 200644 Holstein cattle (50545 heifers, 150099 dairy cows). The data ranges for each parameter were selected based on the previous investigations and dairy farm management strategies.

### Statistical analysis

Dependent variables including AFC, DFS, CI, AFS, DO, SPC, and CR, were analyzed for both cows and heifers. Continuous variables including DO, AFS, AFC, CI, and DFS were analyzed using MIXED procedure of the statistical analysis system (SAS, 2004). The SPC was analyzed with the GENMOD procedure and Poisson distribution. Binomially distributed data such as CR was analyzed by the GENMOD method using a binary distribution and a logit odds ratio link. The model included fixed effects of year, season, herd, and the number of parturitions and their two- and three- ways interactions. The mean of annual milk production throughout the experiment was included in the model as a covariate variable. Also, post-partum disease status (reproduction disease, lameness, mastitis, etc.) and post-partum body condition score (BCS) were included in the model; however, they were removed and data were re-analyzed when their effects were not significant. The results are presented as least squares of means (LSM) and standard error (SE), and Tukey's test was used for multiple comparisons.

## RESULTS AND DISCUSSION

Generally, two- and three-ways interactive effects of herd, year, and season were significant on reproductive parameters of cows and heifers ( $P < 0.01$ ). The reproductive traits in both heifers and dairy cows were affected by year (Tables 1 and 2, respectively). In dairy cows, the DFS declined from 79 d in 2007 to 66 d in 2018 ( $P < 0.05$ ); however, DO fluctuate between 132-141 d during 2007 to 2014, and then remained constant (136 d) until 2018 ( $P < 0.05$ ). There was no significant change in the average CI during the experiment. A 9% decline in CR was recorded over the time from 2007 to 2018, while the SPC increased from 1.94 in 2007 to 2.59 in 2018 ( $P < 0.05$ ). In the heifers, a decreasing trend was recorded in AFS (484 d in 2007 to 465 d in 2018;  $P < 0.05$ ) and in AFC (from 772 d to 758 d;  $P < 0.05$ ) over the time. The CR fluctuated between 80 to 87% over time, whereas it was highest in 2008, 2015, and 2018 and was lowest from 2009 to 2014. Accordingly, the higher CR reduced SPC in 2007, 2008, 2015, and 2018 compared to the other years ( $P < 0.05$ ; Table 2). The herd had a significant effect on DFS, DO, CI, CR, SPC in dairy cows ( $P < 0.05$ ; Table 3). Herd 1 had the longest DFS and DO, while the shortest value was in herd 6 ( $P < 0.05$ ). The CI was ranged 398-446 d among the herds, where the longest CI was observed in herds 1 and 3, herds 5 and 6 had the lowest CI.

**Table 1** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy cows from 2007 to 2018

Years	Reproductive traits				
	DFS	DO	CI	CR	SPC
2007	79±0.54 <sup>ab</sup>	132±1.48 <sup>cd</sup>	414±1.70 <sup>e</sup>	67±0.68 <sup>a</sup>	1.94±0.03 <sup>f</sup>
2008	77±0.45 <sup>bc</sup>	136±1.33 <sup>bcd</sup>	417±1.50 <sup>cde</sup>	65±0.62 <sup>ab</sup>	2.01±0.03 <sup>ef</sup>
2009	80±0.39 <sup>a</sup>	137±1.10 <sup>bc</sup>	421±1.30 <sup>abcd</sup>	64±0.51 <sup>bc</sup>	2.07±0.02 <sup>de</sup>
2010	76±0.37 <sup>c</sup>	141±0.91 <sup>a</sup>	424±1.10 <sup>a</sup>	64±0.43 <sup>bc</sup>	2.06±0.02 <sup>de</sup>
2011	77±0.35 <sup>bc</sup>	136±0.86 <sup>bc</sup>	421±1.04 <sup>abc</sup>	63±0.41 <sup>bc</sup>	2.10±0.02 <sup>d</sup>
2012	72±0.32 <sup>d</sup>	139±0.82 <sup>ab</sup>	421±1.00 <sup>abc</sup>	62±0.39 <sup>e</sup>	2.13±0.02 <sup>d</sup>
2013	70±0.29 <sup>e</sup>	134±0.74 <sup>cd</sup>	416±0.90 <sup>de</sup>	64±0.35 <sup>bc</sup>	2.08±0.02 <sup>d</sup>
2014	65±0.27 <sup>h</sup>	131±0.68 <sup>d</sup>	415±0.83 <sup>e</sup>	64±0.32 <sup>bc</sup>	2.18±0.01 <sup>c</sup>
2015	67±0.27 <sup>j</sup>	136±0.63 <sup>bc</sup>	417±0.76 <sup>cde</sup>	58±0.31 <sup>d</sup>	2.49±0.01 <sup>b</sup>
2016	68±0.25 <sup>f</sup>	136±0.61 <sup>bc</sup>	421±0.75 <sup>ab</sup>	58±0.30 <sup>d</sup>	2.53±0.01 <sup>b</sup>
2017	69±0.24 <sup>e</sup>	136±0.6 <sup>bc</sup>	420±0.74 <sup>abcd</sup>	57±0.31 <sup>d</sup>	2.59±0.01 <sup>a</sup>
2018	66±0.24 <sup>h</sup>	136±0.58 <sup>bc</sup>	418±0.74 <sup>bcd</sup>	58±0.31 <sup>d</sup>	2.59±0.01 <sup>a</sup>

DFS: days from calving to first service; DO: days open; CI: calving interval (d); CR: conception rate (%) and SPC: service per conception. The means within the same column with at least one common letter, do not have significant difference (P>0.05). Note: two- and three-ways interaction effects of herd, year and season on the studied parameters were significant (P<0.01).

**Table 2** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy heifers from 2007 to 2018

Years	Reproductive traits			
	AFS	AFC	CR	SPC
2007	484±1.81 <sup>a</sup>	772±2.22 <sup>ab</sup>	85±1.2 <sup>ab</sup>	1.37±0.03 <sup>bc</sup>
2008	486±1.46 <sup>a</sup>	773±1.92 <sup>a</sup>	87±1.03 <sup>a</sup>	1.32±0.03 <sup>c</sup>
2009	472±1.34 <sup>cd</sup>	762±1.67 <sup>c</sup>	82±0.90 <sup>ab</sup>	1.44±0.03 <sup>abc</sup>
2010	473±1.27 <sup>b</sup>	763±1.60 <sup>bc</sup>	84±0.86 <sup>ab</sup>	1.41±0.03 <sup>abc</sup>
2011	470±0.60 <sup>cde</sup>	758±1.62 <sup>c</sup>	80±0.87 <sup>b</sup>	1.47±0.03 <sup>ab</sup>
2012	470±1.32 <sup>cde</sup>	760±1.82 <sup>c</sup>	81±0.69 <sup>b</sup>	1.49±0.02 <sup>a</sup>
2013	470±1.13 <sup>cde</sup>	762±1.50 <sup>c</sup>	82±0.76 <sup>ab</sup>	1.46±0.02 <sup>ab</sup>
2014	472±0.75 <sup>c</sup>	746±1.38 <sup>d</sup>	85±0.81 <sup>ab</sup>	1.38±0.02 <sup>bc</sup>
2015	456±1.06 <sup>f</sup>	748±1.42 <sup>d</sup>	86±0.74 <sup>a</sup>	1.35±0.02 <sup>c</sup>
2016	441±0.99 <sup>e</sup>	738±1.29 <sup>e</sup>	84±0.37 <sup>ab</sup>	1.41±0.01 <sup>abc</sup>
2017	466±1.02 <sup>de</sup>	760±1.06 <sup>c</sup>	84±0.57 <sup>ab</sup>	1.41±0.02 <sup>abc</sup>
2018	465±1.24 <sup>e</sup>	758±0.69 <sup>c</sup>	85±0.99 <sup>ab</sup>	1.39±0.03 <sup>bc</sup>

AFS: age of first service (d); AFC: age of first calving (d); CR: conception rate (%) and SPC: service per conception. The means within the same column with at least one common letter, do not have significant difference (P>0.05). Note: two- and three-ways interaction effects of herd, year and season on the studied parameters were significant (P<0.01).

**Table 3** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy cows from different herds

No. herd	Reproductive traits				
	DFS	DO	CI	CR	SPC
1	89±0.22 <sup>a</sup>	154±0.46 <sup>a</sup>	445±0.60 <sup>a</sup>	70±0.24 <sup>a</sup>	1.9±0.01 <sup>d</sup>
2	78±0.23 <sup>b</sup>	145±0.67 <sup>b</sup>	421±0.90 <sup>b</sup>	59±0.34 <sup>d</sup>	2.5±0.02 <sup>a</sup>
3	67±0.25 <sup>c</sup>	150±0.50 <sup>a</sup>	446±0.60 <sup>a</sup>	63±0.24 <sup>b</sup>	2.2±0.01 <sup>c</sup>
4	68±0.23 <sup>d</sup>	137±0.71 <sup>c</sup>	403±0.80 <sup>c</sup>	60±0.32 <sup>c</sup>	2.3±0.01 <sup>b</sup>
5	70±0.33 <sup>c</sup>	123±0.96 <sup>d</sup>	398±1.12 <sup>d</sup>	62±0.44 <sup>bc</sup>	2.2±0.02 <sup>c</sup>
6	62±0.15 <sup>f</sup>	123±0.45 <sup>d</sup>	399±0.52 <sup>d</sup>	58±0.21 <sup>d</sup>	2.4±0.01 <sup>a</sup>

DFS: days from calving to first service; DO: days open; CI: calving interval (d); CR: conception rate (%) and SPC: service per conception. The means within the same column with at least one common letter, do not have significant difference (P>0.05). Note: two- and three-ways interaction effects of herd, year and season on the studied parameters were significant (P<0.01).

Herds 2 and 6 had the lowest CR and the highest SPC values compared to the other herds (P<0.05; Table 3). In the case of heifers, the highest and lowest AFS and AFC were observed in herds 1 and 4, respectively (P<0.05). Interestingly, herd 1 had the highest CR and the lowest SPC values (P<0.05; Table 4). The effect of season on the reproductive parameters is presented in Tables 5 and 6.

The average values for DFS, DO, CI, and SPC in cows were lower in winter compared to other seasons (P<0.05; Table 5). The maximum CR was observed in winter and spring in dairy cows (P<0.05; Table 5). In heifers, CR and SPC were not affected by season, but AFS and AFC were increased in summer compared to other seasons (Table 6, P>0.05).

**Table 4** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy heifers from different herds

No. herd	Reproductive traits			
	AFS	AFC	CR	SPC
1	481±0.71 <sup>a</sup>	786±0.81 <sup>a</sup>	86±0.44 <sup>a</sup>	1.34±0.01 <sup>b</sup>
2	467±0.60 <sup>b</sup>	754±0.64 <sup>b</sup>	83±0.35 <sup>c</sup>	1.43±0.01 <sup>a</sup>
3	479±1.54 <sup>a</sup>	759±2.22 <sup>b</sup>	83±0.38 <sup>c</sup>	1.47±0.03 <sup>a</sup>
4	459±0.62 <sup>d</sup>	749±0.67 <sup>c</sup>	84±0.36 <sup>b</sup>	1.41±0.01 <sup>a</sup>
5	463±0.85 <sup>c</sup>	750±0.93 <sup>c</sup>	85±0.50 <sup>ab</sup>	1.36±0.01 <sup>ab</sup>
6	468±0.42 <sup>b</sup>	754±0.46 <sup>b</sup>	84±0.25 <sup>b</sup>	1.42±0.01 <sup>a</sup>

AFS: age of first service (d); AFC: age of first calving (d); CR: conception rate (%) and SPC: service per conception.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

Note: two- and three-ways interaction effects of heard, year and season on the studied parameters were significant ( $P<0.01$ ).

**Table 5** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy cows during different seasons

Seasons	Reproductive traits				
	DFS	DO	CI	CR	SPC
Winter	69±0.19 <sup>c</sup>	133±0.51 <sup>c</sup>	417±0.6 <sup>b</sup>	63±0.24 <sup>a</sup>	2.20±0.01 <sup>c</sup>
Spring	73±0.19 <sup>a</sup>	136±0.58 <sup>b</sup>	418±0.70 <sup>ab</sup>	63±0.28 <sup>a</sup>	2.21±0.01 <sup>c</sup>
Summer	74±0.21 <sup>a</sup>	138±0.51 <sup>a</sup>	420±0.60 <sup>a</sup>	60±0.23 <sup>c</sup>	2.34±0.01 <sup>a</sup>
Autumn	72±0.21 <sup>b</sup>	136±0.57 <sup>b</sup>	420±0.70 <sup>a</sup>	61±0.27 <sup>b</sup>	2.31±0.01 <sup>b</sup>

DFS: days from calving to first service; DO: days open; CI: calving interval (d); CR: conception rate (%) and SPC: service per conception.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

Note: two- and three-ways interaction effects of heard, year and season on the studied parameters were significant ( $P<0.01$ ).

**Table 6** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy heifers during different seasons

Seasons	Reproductive traits			
	AFS	AFC	CR	SPC
Winter	468±0.56 <sup>b</sup>	759±0.71 <sup>b</sup>	84±0.39	1.39±0.01
Spring	469±0.59 <sup>b</sup>	756±0.73 <sup>b</sup>	83±0.38	1.43±0.01
Summer	472±0.60 <sup>a</sup>	761±0.79 <sup>a</sup>	84±0.39	1.40±0.01
Autumn	469±0.58 <sup>b</sup>	757±0.73 <sup>b</sup>	84±0.43	1.40±0.01

AFS: age of first service (d); AFC: age of first calving (d); CR: conception rate (%) and SPC: service per conception.

The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

Note: two- and three-ways interaction effects of heard, year and season on the studied parameters were significant ( $P<0.01$ ).

The THI trends, as an index of the geographical and climatic characteristics of herds, is presented in Figure 1. In winter, spring, and autumn seasons, herds 5 and 6 experienced more THI than other herds. Also, herds 3, 5, and 6 were exposed to more heat stress during the summer.

The number of parturitions had a significant effect on the reproductive traits of dairy cows ( $P<0.05$ ; Table 7). The mean DFS and CR were higher ( $P<0.05$ ) in the first number of parturitions than the others. However, DO, CI and SPC increased as the age of the cow increased up to the sixth the number of parturitions ( $P<0.05$ ).

The trend in the reproductive performance of Iranian Holstein dairy herds has been analyzed in heifers and dairy cows for 11 years.

As critical factors affecting reproduction in dairy cows, the effect of herd, year, season, and the number of parturitions were analyzed. Mean values for the reproductive parameters indicated suboptimal reproductive performance in the studied herds in Iran which is similar to most other countries worldwide.

In the current study, the mean interval of DFS in Holstein dairy cows was 69 days. It had a significant reduction from 79 d in 2007 to 66 d in 2018.

This is lower than the previously reported values of 74 days (Eghbalsaid, 2011) in Iran, 85-92 days in the USA (Norman *et al.* 2009), 80 days in Ireland (Berry and Cromie, 2009) and, 71 days in Ireland (Mee, 2004), but, is similar to a previous report by Ansari-Lari *et al.* (2010) for Iranian Holstein cows. The discrepancy between studies may be derived from the level of milk production of the investigated herds. However, the decrease in DFS is likely due to the increased use of synchronization protocols in dairy farms. The use of hormonal programs has improved the efficiency of heat detection in a shorter and more predictable timeframe and resulted in a shortened DFS (Norman *et al.* 2009). The DFS is influenced by the efficiency of estrus detection method, the VWP and the metabolic and hormonal status of the animal before returning to estrus cycle (Caraviello *et al.* 2006). An indication of good fertility management is that 95% of cows must be having an average of DFS less than 70 days (Yusuf *et al.* 2011). Herd 6 had the lowest DFS (62 d), whereas herd 1 had the greatest DFS (89 d). These differences may be caused by geographic location and weather conditions (Figure 1) which in turn affect estrus expression and response of the cows to reproductive management.

**Table 7** Reproductive traits (Least squares mean±Standard error of the mean) in Iranian Holstein dairy cows according to the number of parturitions

The number of parturitions	Reproductive traits				
	DFS	DO	CI	CR	SPC
1	76±0.14 <sup>a</sup>	134±0.41 <sup>c</sup>	419±0.50 <sup>ab</sup>	65±0.17 <sup>a</sup>	2.13±0.01 <sup>c</sup>
2	70±0.16 <sup>d</sup>	132±0.36 <sup>d</sup>	416±0.58 <sup>c</sup>	62±0.20 <sup>b</sup>	2.21±0.01 <sup>b</sup>
3	70±0.18 <sup>d</sup>	134±0.48 <sup>c</sup>	415±0.43 <sup>c</sup>	62±0.23 <sup>b</sup>	2.22±0.01 <sup>b</sup>
4	71±0.23 <sup>c</sup>	135±0.59 <sup>bc</sup>	421±0.92 <sup>ab</sup>	62±0.29 <sup>b</sup>	2.24±0.01 <sup>ab</sup>
5	72±0.29 <sup>b</sup>	138±0.75 <sup>b</sup>	420±0.73 <sup>b</sup>	61±0.37 <sup>bc</sup>	2.38±0.02 <sup>a</sup>
6	73±0.38 <sup>b</sup>	142±1.00 <sup>a</sup>	424±1.25 <sup>a</sup>	60±0.50 <sup>c</sup>	2.30±0.02 <sup>a</sup>

DFS: days from calving to first service; DO: days open; CI: calving interval (d); CR: conception rate (%) and SPC: service per conception. The means within the same column with at least one common letter, do not have significant difference ( $P>0.05$ ).

Note: two- and three-ways interaction effects of herd, year and season on the studied parameters were significant ( $P<0.01$ ).

DO in the last three years (2016-2018) followed a decreasing trend, indicating a significant improvement in reproductive management resulted in higher fertility and conception rates. The data analysis also showed a significant effect of the herd on DO, where the longest DO was recorded in herds 1 and 3, and the shortest values were recorded in herd 5 and 6. Such differences could be attributed to the difference in observed heat estrus which affected by the level of negative energy balance, the manifestation of reproductive behavior and VWP applied by the farmers. Given the VWP was almost in a narrow range between herds, the later factors are probably more involved. It has been shown that the main cause of a lengthened DO in dairy cattle is poor estrus detection efficiency (Motlagh *et al.* 2013). Interestingly, season had also affected the DO. The highest DFS and DO were observed in the summer and spring, and the lowest DFS was in the winter. Season did not affect CR in heifers, but in cows, it was higher during spring and winter when they had a lower SPC. In comparison to dairy cows, heifers do not experience postpartum negative energy balance and metabolic disorders as well as reproductive tract infection. The similar trend observed in the DFS of heifers and cows indicates that the main influential factors are related to heat stress. It has been suggested that higher temperature during summer changes the concentration of reproductive hormones and leads to a longer DFS (Wolfenson *et al.* 2000).

The present findings revealed that the greatest DFS was observed in the first number of parturitions probably due to the higher growth requirements (Yalew *et al.* 2011; Maciel *et al.* 2012). It is worth noting that as compared with the second and third parturitions, DFS was significantly longer in the fifth and sixth parturition cows. The shortest DO was also recorded for the second number of parturitions. This is in agreement with the findings of, Dobson *et al.* (2007), Chagas *et al.* (2007) and Ansari-Lari *et al.* (2010). DO is commonly used to evaluate reproductive performance and to make an economic decision in dairy farms (Farin *et al.* 1994). Generally, by increasing the number of parturitions, the environmental sensitivities of the animal increases and the uniformity of performance in the herd decrease.

Similarly, increasing the number of parturitions, when is combined with the negative energy balance during postpartum period, increases the possibility of reproductive problems and lowers CR (Tenhagen *et al.* 2003). In agreement with these findings, Yalew *et al.* (2011) showed that cows with first and fifth parity had longer DFS. The longer DO in the cows with sixth parity reported in this study is partially due to the higher incidence of reproductive problems such as retained placenta, ovarian cysts (data were not shown).

In this study, the mean CI was  $419 \pm 83$  d and the overall average DO was 136 d was consistent with the previous reports for Holstein cows in Iran (Bahonar *et al.* 2009; Ansari-Lari *et al.* 2010). However, it is longer than the optimal value of 400 and 117 days which was respectively reported for CI and DO in dairy farms with appropriate reproductive management (González-Recio and Alenda, 2005; Hare *et al.* 2006; Atashi *et al.* 2012). The CI is included of DO and pregnancy period. Given that the length of pregnancy period usually does not change much, DO should be the main factor affecting the CI. This component has a high correlation with CI and it is a criterion that with its reduction, the desirable CI will be obtained (Miller *et al.* 2007). As the DO is strictly associated with health management during transition period as well as postpartum reproductive strategies, it could be expected that by controlling the negative energy balance and metabolic disorders during transition period and postpartum reproductive disease, the CI will be shortened. In addition, postpartum ovarian resumption and uterus involution which can reduce postpartum time needed for cyclicity resumption has a great impact on DO and consequently CI.

As a measure of reproductive performance in dairy cattle, CR is directly related to pregnancy rate (Chebel *et al.* 2004). On the other hand, CR is negatively associated with SPC. CR for cows (59%) was lower than heifers (83%) during the evaluated period. This is, however, higher than the estimates reported by Ansari-Lari *et al.* (2010) for the same breed in Iran (41%). It has been shown that the average CR for heifers is higher than that of cows (Washburn *et al.* 2002). Based on these findings, the number of SPC needed for heifers was less than cows (63%).

The average number of SPC in this study was similar to Jamrozik *et al.* (2005) and Eghbalsaied (2011), which were 1.64 for heifers and 2.14 and 2.27 for cows, respectively. Probably high milk production, the negative energy balance as well as more susceptibility to environmental conditions are the main reasons for the increased SPC and the reduced CR values in cows than heifers (Washburn *et al.* 2002; Tesfaye *et al.* 2015).

The highest CR and SPC among cows were observed in 2007 and 2008 for heifers. The trend of changes in CR in cows declined markedly from 2015 to 2018 and reached 58%. From 2008 to 2018, changes in the number of SPC were remarkable and ranged from 2 to 2.6. Although part of this decline may be due to the increased milk production, it is possible that other factors such as the type of inseminated sperm, or negative energy balance affected this trait. Overall, a sinusoidal change accompanied by increases and decreases was observed in the Holstein CR during the study period. The number of parturitions had also affected the fertility parameters. The highest and lowest CR was observed in the first and sixth parity, respectively. This is in agreement with the findings of Irikura *et al.* (2018), which showed that CR decreased by increasing the number of parturitions. Thus, considering the negative relationship between CR and SPC, the highest number of SPC in cows was observed after fourth parturitions, while cows with the first parity with a value of 2.13 for SPC had the best performance. It could be attributed to the relative decrease in reproductive performance. It has been argued that older cows are more likely to experience periparturient problems leading to reduced fertility (Chebel *et al.* 2004).

The significant effect of season on SPC in this study was in agreement with the results of Asimwe and Kifaro (2007). It has been widely accepted that heat stress reduces reproductive performance by affecting feed intake, negative energy balance, steroid hormones production, oocyte developmental competence and decreasing estrus signs (Vanselow *et al.* 2016; Abazarikia *et al.* 2020). Long-term heat stress in summer reduces the amount of endogenous production of steroid hormones by theca cells, resulting in decreased follicular fluid estradiol concentration and reduced fertility (Reist *et al.* 2003). Studies have shown that heat stress in the summer and continuation of heat stress effects in the autumn are negatively associated with CR (Wu *et al.* 2000; De Rensis and Scaramuzzi, 2003). It has also been suggested that high ambient temperature during estrus has a negative correlation with the CR and a positive correlation with the SPC in dairy cows (Ingraham *et al.* 1976; Hammoud *et al.* 2010). In harmony with other parameters, mean AFS and AFC in summer-born calves was significantly higher than other seasons. These results are consistent with the study of Fekadu *et al.* (2011) who attributed

such changes to the impact of environmental conditions on growth and maturity of the calves. It has been shown that prepubertal growth and development of calves are affected by the variability of the quantity and quality of feeds in various seasons which in turn affect the AFS (Asimwe and Kifaro, 2007; Chebel *et al.* 2007). Also, it seems that due to the management and more knowledge of heifers breeding in recent years, AFS and AFC have decreased.

## CONCLUSION

In conclusion, results from the current study showed that the parameters such as CI, DO, SPC have been increased and traits such as DFS, CR, AFS, and AFC decreased over time. Calving in the summer and autumn had been lower compared to winter and spring. The best reproductive performance was related to the second and third number of parturitions, and after fourth parturitions reduced due to age-related subfertility. These changes in reproductive parameters may be due to increment of metabolic and environmental stressors which in turn disturb biology of reproductive system. The results of the present study emphasize on need for applying more efficient strategies to improve reproduction performance in Iranian dairy cows, especially during summer and after third parturition. It is worth noting that some parameters such as SPC showed a significant difference even though in small changes between groups. These probably were occurred due to the high number of data used in this study which in turn reduced standard error. However, more investigations are needed to figure out whether these small changes are biologically significant.

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