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Original Research Article

**A retrospective study on the relationship among different dry period lengths, udder health status and their possible effects on the reproductive performance of Holstein-Frisian cows**

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**ABSTRACT**

This study aimed to evaluate the effect of different dry period lengths (DPLs) on the udder health and fertility during the subsequent lactation. The impact of subclinical mastitis (SCM) and clinical mastitis (CM) on the reproductive efficiency were also investigated. Holstein-Friesian cows (n=894) were included in the study of udder health, of which only multiparous cows (n=499) were included in the investigation of the impact of different DPLs on the udder health and reproductive efficacy in the subsequent lactation. Cows were classified according to the DPLs into 3 groups: Short (SDPL, <40 d), Traditional (TDPL, 40-60 d) and Long (LDPL, >60 d). A limit of 200,000 somatic cell/mL milk was established to separate between healthy and mastitic udder status. Accordingly, samples with 500,000-1,000,000 cell/ml were referred to cows affected with SCM and samples with >1,000,000 cells/ml were mentioned to cows affected with CM. A higher proportion of infected udder was detected in cows with LDPL (39.19%) Moreover, a higher proportion of SCM was recorded for cows with LDPL (17.57%) compared with that recorded for cows with SDPL (8.0%) and TDPL (6.13%). A higher proportion of CM was recorded for cows with a long dry period (LDP, 8.11%) compared with that recorded for cows with SDPL (4.0%) or with TDPL (4.8%). Cows with SDPL showed the best intervals reproductive indices compared with that recorded for both TDPL and LDPL. Furthermore, LDPL was found to be associated with significantly lower CR (33.11±2.91%), lower PR (50.39±3.97%) and higher NSPC (1.98±0.37) compared with values that recorded for either SDPL or TDPL. Cows with either SCM or CM showed the longest interval reproductive indices, less CR and PR and number of inseminations to become pregnant compared with healthy udder cows. A lower proportion of affected udder was recorded for Primiparous cow (24.3%) compared with that showed by multiparous cows (32.46%). Cows affected with mastitis during the voluntary waiting period (VWP) showed longer intervals to the first detected estrus and first services while those affected during the service period (SP) showed longer calving to conception intervals, lower CR and lower PR especially when the SCC exceed 500,000 cell/ml milk. Cows experienced udder infection during the SP showed the lowest PR (32.54% & 36.44%) and required more NSPC. In conclusion, a significant linkage among different dry period lengths and both the incidence of

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mastitis and fertility were recorded, with the best results were recorded for cows with TDPL and SDPL. Extending the dry period above 60 days increased the incidence of both clinical and subclinical mastitis and exerted a negative impact on the reproductive performance. The occurrence of mammary infection during the VWP increased the calving to first estrus and first service intervals, while its occurrence during the SP prolonged the days open, and extremely reduced the conception and pregnancy rates.

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## 1. INTRODUCTION

Reproductive efficiency plays a great role in the dairy industry as it is considered one of the greatest factors associated with dairy farm profitability. Reproductive efficacy was closely related to uterine, claw and udder health status. A strong linkage between reproductive efficiency and udder health was documented, as the high incidence of mastitis was associated with increased days to first service, number of services per conception (NSPC), days open (DO), embryonic loss and decreased PR (Moore et al., 2005; Ahmadzadeh et al., 2009; Pinedo et al., 2009; Lavon et al., 2011; Hudson et al., 2012). subclinical mastitis (SCM) is defined as an intramammary infection and is commonly diagnosed based on increased milk somatic cell count (SCC) ( $\geq 200,000$  SCC/ml milk) (Gómez-Cifuentes et al., 2014). The greatest harmful effects of SCM on reproduction potentials were observed when it occurred near insemination or during the interval between first insemination and pregnancy diagnosis (Schrick et al., 2001; Lavon et al., 2011; Hudson et al., 2012). There are conflict results about the effect of dry period length (DPL) on the udder health. Some researchers stated a lower SCC in the next lactation following a short (<30-d) DP (Pezeshki et al., 2007; Pinedo et al., 2011). Other researchers found no effects (Rastani et al.,

2005; Church et al., 2008) while others showed a tendency to increase SCC after a short dry period (SDP) compared with traditional dry period (TDP) (60-d) (Kuhn et al., 2006; Pezeshki et al., 2007). The ideal duration of the DP has been an issue of discussion, with a period of 51 to 60 d given as the conventional recommended length (Church et al., 2008). Moreover, it had been estimated that the most advantageous dry periods were ranged from 40 to 60 days (Soleimani et al., 2010; Sawa et al., 2012). It had been estimated that, reproductive performance was improved when the DP is shortened from 55 to 34 d (Watters et al., 2009). However, another study found no improvement in first service CR and services per conception after shortening the DP from 49 to 28 d (Pezeshki et al., 2008). As a consequence of tenuous research that has been done on the effect of DPL on the udder health status and reproductive efficacy, the objectives of this study were to explore the linkage between DPL, udder health status and their possible influence on the reproductive efficiency in the next lactation in Holstein dairy cows.

## 2. MATERIAL AND METHODS

### 2.1. Animals

Over two years (from May, 2017 till April, 2019), a total number of 894 Holstein-Friesian cows (395 Primiparous; 499 multiparous) were included in the present study. Cows were reared

in a large commercial dairy farm located in the northwest region in Giza/Egypt. Reproductive, productive and health data of these cows were recorded.

## **2.2. Housing, Nutrition and Milking**

Cows were reared in open yard system and were allocated according to the stage of lactation and daily milk production. Cows were milked thrice daily in a milking parlor furnished with an automated milking machine. Cows were fed a totally mixed ration (TMR) according to NRC (2001) and had free access to water. Teat dipping was routinely performed at milking and milking machines were backflushed after removal from cows.

## **2.3. Dry off Protocol and Nutritional Regime**

All cows were dried off approximately 8 weeks before expected calving. The transition of the cows to be dried to the dry cow ration at d 7 before the actual drying-off day and transition to milking once daily at d 4 before the actual drying-off day. At drying off, cows received intramammary treatment with a dry cow antibiotic preparation contained 314 mg of potassium benzylpenicillin, 1,000 mg of procaine benzylpenicillin, and 500 mg of neomycin sulfate (Supermastidol, Virbac Animal Health, Barneveld, and the Netherlands) approved for use in non-lactating cows following the last milking. Early dry cows were fed on the far-off ration with low energy. Dried cows received a low-energy far-off diet during the first 5 weeks of the dry period and is intended to preserve body condition of the cow, whereas a moderate-energy close-up diet is presented during the final 3 weeks of the dry period and is planned for adaptation of rumen microorganisms to the high-energy fresh lactation diet provided immediately after parturition till 21 days in milk (DIM) and a high energy lactation diet was offered for the remainder of the lactation period according to

the level of milk production. Cows with short dry periods included those with short gestation period, spontaneously aborted, and cows with incorrect insemination date. Meanwhile, cows with long dry periods were those of long days open and prolonged milk production.

## **2.4. Milk Sampling**

Individual bulk milk samples belonging to definite yards were firstly taken from different milk tanks for the detection of bulk SCC by using the DeLaval cell counter (DeLaval AMR, Tumba, Sweden). Cows belonging to bulk tank samples with SCC > 200,000/ml milk were examined individually for SCC. Two milk samples were aseptically collected from each individual suspected cows in sterile plastic vials three days after calving, and then twice a month for the first three months. Samples were kept in the refrigerator and submitted for SCC analysis and bacteriological examination. According to the modified classification of Sawa et al. (2015), cows were categorized into 1) cows with good, normal healthy udder (<200,000 SCC/mL milk), 2) cows with minatory (threatened and latent changes) udder (200,000-500,000), cows with subclinical mastitic udder (>500,000–1,000,000 SCC/mL milk) and 3) cows with clinical mastitis (>1,000,000 SCC/mL milk).

## **2.5. Reproductive Parameters and Management**

After a voluntary waiting period of 50 DIM, all cows were observed for estrus three times daily and those detected in standing estrus were artificially inseminated by well-trained AI technicians based on am/pm rules. Pregnancy diagnosis was performed at 35-40 days after insemination using a Real Time B-Mode Ultrasonic equipped with 7.5-MHz transrectal transducer (Easi-Scan; BCF Technology, Rochester, MN, USA).

Reproductive performance was evaluated by the following outcome variables:

- (1) CEI: the number of days between calving and the subsequent first detected estrus.
- (2) CSI: the number of days between calving and the subsequent first service.
- (3) CCI: the number of days between calving and the insemination that resulted in pregnancy.
- (4) CR1: the conception rate of the first postpartum insemination.
- (5) PR: Pregnancy rate within 150 days postpartum.
- (6) NSPC: the number of services that a cow required to become pregnant during the 150 DIM.

### 2.6. Data Collection

Only records of cows with complete reproductive data were taken in consideration. With this edit, only 499 multiparous cows were included in the investigation of effect of DPL on udder health and fertility, while for studying the effect of udder health status on the reproductive efficiency a total of 894 cows (395 primiparous and 499 multiparous) were included. All information about each individual cow such as: identification, birth date (age), parity, body conditions scores (BCS), days in milking, average milk yield at the day of sampling, calving date, breeding date, pregnancy, abortion, date of drying off, health and metabolic disorders, number of previous inseminations were obtained from the farm records.

### 2.7. Statistical analysis

Out of 894 Holstein-Frisian, cows only 499 multiparous cows were included in the statistical analysis to investigate the effect of different DPLs on both udder health and fertility in the following lactation. Meanwhile, for investigation the effect of udder health status on the reproductive efficiency, the data of 894 cows (395 primiparous and 499 multiparous) were

include in the statistical analyses. Dry period lengths were categorized to three classes (short < 40 days, traditional (40-60days) and long (> 60 days). All data were primarily entered into an Excel spreadsheet (Microsoft office 2016), and then transferred to SPSS version 25 (IBM, SPSS Inc., Chicago, IL, USA) for data cleaning, coding and analysis. Differences in, CR, PR within 150 among the different groups of cows were analyzed using Chi-square test. Likewise, the association between the different DPLs and the different levels of SCC were also analyzed. Using 2 way ANOVA. When groups differed ( $P \leq 0.05$ ), post-hoc test was used to compare between individual groups. Furthermore, the differences in CEI, CSI, CCI among the groups with different degrees of SCC or with different DPLs were analysed using one-way ANOVA.

### 3. RESULTS

As depicted in Table 1, about 75.15% (375/499) of multiparous Holstein-Friesian cows had a TDPL ranged from 40 to 60 days. About 14.82% (74/499) of cows were found to have LDPL (>60 days) and only 10% (50/499) of cows with SDPL (<40 days). The proportion of multiparous cows with healthy udder (<200,000 SCC/ml milk) was found to be 67.54% (337/499) and only 32.46% (162/499) of multiparous cows showed different degree of intramammary affection (>200,000 SCC/ml milk) divided into cows with a threatened udder (19.24%), cows with SCM (8.02%) and cows with CM (5.21%). There was a close relationship between the DPL and postpartum udder health, as the intramammary infection tended to be higher in cows with LDPL (39.19%) compared with SDPL (28.0%) and TDPL (31.73%). Moreover, a higher percentage of milk samples indicative of good udder health (<200,000 SCC/ml) was observed in cows with SDPL (72.0%), and a lower percentage (60.81%) was observed in cows with LDPL. A higher proportion of milk samples that indicative of SCM was recorded for cows with LDPL (17.57%) compared with that



recorded for cows subjected to SDPL (8.0%) and TDPL (6.13%). It was noticeable that elongation of the DP resulted in significantly ( $p<0.05$ ) higher proportion (8.11%) of cows affected with

CM compared with that observed for cows with SDPL (4.0%) or with TDPL (4.8%).

**Table 1. Distribution of multiparous Holstein-Friesian cows according to the DPL and udder health**

Distribution of cows according to DPL (%)		Distribution of cows according to SCC score (%)			
		Healthy (n=337)	Affected Udder (n=162)		
			Minatory (n=96)	SCM (n=40)	CM (n=26)
<b>SDPL (n=50)</b>	10.02 <sup>a</sup> (50/499)	72.00 <sup>a</sup> (36/50)	16.00 <sup>a</sup> (8/50)	8.00 <sup>b</sup> (4/50)	4.00 <sup>a</sup> (2/50)
			28 (14/50)		
<b>TDPL (n=375)</b>	75.15 <sup>b</sup> (375/499)	68.27 <sup>a</sup> (256/375)	20.80 <sup>a</sup> (78/375)	6.13 <sup>a</sup> (23/375)	4.80 <sup>a</sup> (18/375)
			31.73 (119/375)		
<b>LDPL (n=74)</b>	14.83 <sup>a</sup> (74/499)	60.81 <sup>a</sup> (45/74)	13.51 <sup>a</sup> (10/74)	17.57 <sup>b</sup> (13/74)	8.11 <sup>a</sup> (6/74)
			39.19 (29/74)		

Values with different superscript letters in the same column are significantly different ( $p<0.05$ ); SCC: Somatic cell count; DPL: Dry period length; SDP: Short dry period (<40 days); TDP: Traditional dry period (40-60 days); LDP: long dry period (>60 days); Healthy: normal healthy udder (<200,000SCC); Minatory: A Threatened udder (200,000-500,000 SCC); SCM: Subclinical mastitic udder (500,000-1,000,000 SCC); CM: Clinically mastitis udder (>1,000,000 SCC).

Data presented in Table 2 showed that, multiparous cows with SDPL had the best interval reproductive indices compared with that showed by the TDPL and LDPL groups. Cows with SDPL showed shorter: calving to first estrus (83.67±3.92 d), calving to first service (107.28±4.26d), calving to conception (182.55±4.31d) intervals compared with values that recorded for both TDPL (98.32±3.06d, 133.39±3.48d and 233.32±6.76d respectively) or LDPL (155.17±4.44d, 193.23±5.65d and 281.51±5.23d respectively). It was clear that, LDPL was associated with significantly lower first services CR (33.11±2.91%), lower PR (50.39±3.97%) within 150 DIM and higher NSPC (1.98±0.37) compared with values that recorded for either SDPL (38.19±2.82%, 58.32±3.09%, 1.71±0.11) or TDPL

(43.16±2.56%, 63.35%±3.08, 1.58±0.13 respectively). A close

relationship between udder health and reproductive efficiency was observed in our study. The best reproductive values were recorded for cows with healthy udder (<200,000 SCC/ml) compared with values that recorded for cows either affected with SCM (500,000-1,000,000 SCC/ml) or with CM (>1,000,000 SCC/ml). Cows with CM required more days to come in detected estrus (126.74±3.77d), more days to artificially inseminated (172.2±6.48d), more days to conceive after first AI (240.56±4.68d) and more inseminations to become pregnant (2.69±0.09) compared with healthy udder cows. Moreover, clinically mastitic cows showed the lowest first service

CR (21.91±2.12%) and the lowest 150DIM pregnancy rate (37.15±3.36%). Nearly the same

trend was recorded for cows suffered from SCM.

**Table 2. Dry period lengths and milk somatic cell score and their effects on the reproductive indices in the subsequent lactation of Holstein-Frisian cows.**

RI	Multiparous Cows according to DPL (n=499)			Udder status according to SCC score (n=894)				P values		
	SDP (n=50)	TDP (n=375)	LDP (n=74)	Healthy (n=611)	Minatory (n=142)	SCM (n=98)	CM (n=43)	DP L	SC C	DP L* SC C
<b>CEI (day)</b>	83.67 <sup>ac</sup> ±3.92	98.32 <sup>ad</sup> ±3.06	155.17 <sup>b</sup> ±4.44	78.41 <sup>e</sup> ±4.29	90.27 <sup>ac</sup> ±4.62	111.26 <sup>cd</sup> ±5.1	126.74 <sup>c</sup> ±3.77	.046	.023	.052
<b>CSI (day)</b>	107.28 <sup>a</sup> ±4.26	133.39 <sup>bc</sup> ±3.48	193.23 <sup>c</sup> ±5.65	97.69 <sup>a</sup> ±3.62	123.76 <sup>b</sup> ±4.33	150.37 <sup>ef</sup> ±6.92	172.20 <sup>cf</sup> ±6.48	.035	.011	.032
<b>CCI (day)</b>	182.55 <sup>a</sup> ±4.31	233.32 <sup>bc</sup> ±6.76	281.51 <sup>d</sup> ±5.23	179.56 <sup>a</sup> ±8.7	209.11 <sup>b</sup> ±7.66	236.48 <sup>c</sup> ±5.14	240.56 <sup>c</sup> ±4.68	.008	.241	.063
<b>CR1 (%)</b>	38.19 <sup>ab</sup> ±2.82	43.16 <sup>bc</sup> ±2.56	33.11 <sup>ab</sup> ±2.91	48.74 <sup>c</sup> ±2.28	39.47 <sup>abc</sup> ±2.73	32.94 <sup>a</sup> ±2.92	21.91 <sup>d</sup> ±2.12	.019	.010	.057
<b>PR (%)</b>	58.32 <sup>ac</sup> ±3.09	63.35 <sup>ab</sup> ±3.08	50.39 <sup>ad</sup> ±3.97	73.48 <sup>b</sup> ±3.27	65.42 <sup>bc</sup> ±3.81	51.29 <sup>ac</sup> ±3.67	37.15 <sup>d</sup> ±3.36	.053	.028	.042
<b>NSPC (150DIM)</b>	1.71 <sup>a</sup> ±0.11	1.58 <sup>a</sup> ±0.13	1.98 <sup>ab</sup> ±0.37	1.36 <sup>a</sup> ±0.22	1.53 <sup>a</sup> ±0.15	1.86 <sup>a</sup> ±0.20	2.69 <sup>b</sup> ±0.09	.137	.051	.069

Values with different superscript letters in the same row are significantly different (p<0.05); RI: Reproductive indices; DPL: Dry period length; SDP: Short dry period (<40 d); TDP: Traditional dry period (40-60 days); LDP: long dry period (>60 d); CEI: Calving to first estrus interval; CSI: calving to first service interval; CCI: Calving to conception interval; CR1: Conception rate of the first postpartum insemination; PR: pregnancy rate within 150 days in milk; NSPC: number of services per conception.

Data presented in Table 3 showed a clear association between parity and SCC score. Primiparous cow showed a higher proportion (75.70%) of milk samples with <200,000 SCC indicative a good healthy udder and lower proportion (24.3%) of milk samples with ≥200,000 SCC (P < 0.01) compared with that showed by multiparous cows (67.54% and 32.46% respectively). As declared in Table 3, primiparous cows affected with CM during the VWP (i.e. before the first insemination) required more days (125.17±12.34) to come into detected heat and longer calving to first services interval (182.12±8.34) while those affected during the SP showed drastic effects on calving to

conception interval (255.25±5.95), CR (18.91±3.31%) and PR (32.54±2.53%). As presented in Table 3, longer intervals to first postpartum observed estrus were recorded for multiparous cows experienced either SCM (135.04±5.12d) or CM (134.87±6.86d) during the VWP and SP respectively. The interval from calving to first service was longer for multiparous cows having either SCM (192.19±4.36d) or CM (173.46±5.73) during the VWP than for other cows. The longest DO (calving to conception interval) were recorded for multiparous cows affected either with CM (293.91± 5.36d) or with SCM (287.97±3.98d) during the SP. Primiparous cows suffered from

CM showed the lowest first service CRs when mastitis occurred either during the SP (18.91±3.31%) or during the VWP (23.74±3.34%). Multiparous cows affected with CM showed lower CR either experienced udder infection during the VWP (23.49±2.67%) or SP

(21.51±3.43%). Multiparous cows affected with CM also showed lower PR either udder infection occurred during the SP (36.44%) or VWP (39.35±4.65) and required more NSPC (2.74±0.33).

**Table 3. Udder health status depending on parity and time of SCC elevation and their effects on the reproductive indices in lactating Holstein-Friesian dairy cows**

Parity	SCC	Occurrence	n	Reproductive Indices						
				CEI	CSI	CCI	CR1	PR (150DIM)	NSPC (150DIM)	
Primiparous	Healthy 75.7% (299/395)	VWP	23	69.89 <sup>a</sup> ±4.78	97.89 <sup>ab</sup> ±3.37	160.73 <sup>a</sup> ±3.23	43.97 <sup>a</sup> ±3.98	77.67 <sup>a</sup> ±4.88	1.29 <sup>a</sup> ±0.11	
		SP	276	66.86 <sup>a</sup> ±4.98	94.86 <sup>ac</sup> ±2.08	164.12 <sup>a</sup> ±4.35	43.76 <sup>a</sup> ±3.45	75.97 <sup>a</sup> ±5.08	1.32 <sup>ab</sup> ±0.19	
	Minatory 12.15% (48/395)	VWP	9	78.51 <sup>a</sup> ±8.72	118.46 <sup>b</sup> ±8.72	188.53 <sup>b</sup> ±4.26	39.53 <sup>ab</sup> ±5.45	73.83 <sup>a</sup> ±6.10	1.35 <sup>ab</sup> ±0.37	
		SP	39	75.89 <sup>a</sup> ±5.22	110.98 <sup>bc</sup> ±4.21	210.32 <sup>b</sup> ±7.86	32.54 <sup>abc</sup> ±3.45	61.89 <sup>ab</sup> ±5.08	1.62 <sup>abc</sup> ±0.23	
	SCM 8.86% (35/395)	VWP	7	98.78 <sup>ab</sup> ±9.99	122.33 <sup>bc</sup> ±4.99	198.92 <sup>b</sup> ±5.25	33.86 <sup>abc</sup> ±3.43	52.98 <sup>bc</sup> ±3.42	1.89 <sup>bc</sup> ±0.19	
		SP	28	89.04 <sup>a</sup> ±6.33	114.04 <sup>bc</sup> ±6.33	255.25 <sup>c</sup> ±5.95	30.71 <sup>bc</sup> ±2.45	45.41 <sup>c</sup> ±2.97	2.20 <sup>cd</sup> ±0.15	
	CM 3.29% (13/395)	VWP	4	125.17 <sup>b</sup> ±12.34	182.12 <sup>d</sup> ±8.34	194.37 <sup>b</sup> ±9.07	23.74 <sup>cd</sup> ±3.34	40.25 <sup>cd</sup> ±4.07	2.48 <sup>cd</sup> ±0.37	
		SP	9	123.46 <sup>b</sup> ±5.90	178.37 <sup>d</sup> ±6.13	230.87 <sup>d</sup> ±6.08	18.91 <sup>d</sup> ±3.31	32.54 <sup>d</sup> ±2.53	3.07 <sup>d</sup> ±0.26	
	Multiparous	Healthy 67.54% (337/499)	VWP	28	87.36 <sup>a</sup> ±5.82	99.74 <sup>a</sup> ±5.42	195.09 <sup>b</sup> ±6.6	53.85 <sup>e</sup> ±4.15	71.85 <sup>a</sup> ±5.98	1.39 <sup>ab</sup> ±0.24
			SP	309	89.51 <sup>a</sup> ±3.67	98.27 <sup>a</sup> ±2.91	198.32 <sup>b</sup> ±3.25	53.37 <sup>e</sup> ±3.25	68.44 <sup>a</sup> ±2.60	1.46 <sup>ab</sup> ±0.15
Minatory 19.24% (96/499)		VWP	19	89.89 <sup>a</sup> ±3.22	143.26 <sup>f</sup> ±3.32	217.12 <sup>d</sup> ±3.07	44.93 <sup>a</sup> ±2.74	65.27 <sup>ab</sup> ±3.68	1.53 <sup>ab</sup> ±0.13	
		SP	77	116.78 <sup>b</sup> ±3.19	122.14 <sup>e</sup> ±4.97	220.45 <sup>d</sup> ±3.99	40.87 <sup>ab</sup> ±2.41	60.67 <sup>ab</sup> ±4.37	1.65 <sup>abc</sup> ±0.26	
SCM 8.02% (40/499)		VWP	11	135.04 <sup>b</sup> ±5.12	192.19 <sup>d</sup> ±4.36	203.76 <sup>b</sup> ±3.87	39.32 <sup>ab</sup> ±3.45	60.41 <sup>ab</sup> ±6.43	1.66 <sup>abc</sup> ±0.25	
		SP	29	122.17 <sup>b</sup> ±5.45	170.12 <sup>d</sup> ±3.42	287.97 <sup>e</sup> ±3.98	27.87 <sup>cd</sup> ±2.83	46.37 <sup>b</sup> ±3.22	2.15 <sup>bce</sup> ±0.11	
CM 5.21% (26/499)		VWP	9	123.46 <sup>b</sup> ±4.90	173.46 <sup>d</sup> ±5.73	243.07 <sup>cf</sup> ±6.51	23.49 <sup>cd</sup> ±2.67	39.35 <sup>cd</sup> ±4.65	2.54 <sup>de</sup> ±0.21	
		SP	17	134.87 <sup>b</sup> ±6.86	157.86 <sup>df</sup> ±6.18	293.91 <sup>e</sup> ±5.36	21.51 <sup>cd</sup> ±3.43	36.44 <sup>cd</sup> ±4.12	2.74 <sup>de</sup> ±0.33	

Values with different superscript letters in the same column are significantly different (p<0.05); VWP: voluntary waiting period; SP: service period; SCC: Somatic cell count; CEI: Calving to first estrus interval; CSI: Calving to first service interval; CCI: Calving to conception interval; CR1: Conception rate of the first postpartum insemination; PR (150 DIM): pregnancy rate within 150 days in milk; NSPC

(150DIM): number of services per conception within 150 days in milk. Healthy: good healthy udder (<200,000 SCC); Minatory: A threatened udder (200,000-<500,000 SCC/ml milk); SCM: Subclinical mastitis (500,000-1,000,000 SCC); CM: Clinical mastitis (>1,000,000 SCC/ml milk).

#### 4. DISCUSSION

The dry period is an important time for: proliferation of the udder epithelial cells, preparation of the body for the developing fetus and attaining good postpartum reproductive performance (Church et al., 2008). Data of the present study showed high proportion (75.15%) of Holstein-Friesian cows with a DPL ranged from 40 to 60 days and 14.82% of cows were found to have LDPL (>60 days) and only 10% of cows with a DPL less than 40 days. These results come in agreement with previous studies (Church et al., 2008; Sawa et al., 2012) which suggest that, a dry period of 40–60 days is the most suitable for high milk production and efficient reproduction in the subsequent lactation. In the present investigation the extreme short and extreme long dry periods were not planned, so the most proportion of cows included in this study had traditional dry period.

In the present study, udder health was clearly ( $p<.05$ ) affected by the DPL, as a greater proportion (39.19%) of milk samples indicative to mastitic udder (>200,000 SCC/ml) was detected in cows with LDPL compared with SDPL (28.0%) and TDPL (31.73%) cow groups. A higher proportion of milk samples that indicative of SCM was recorded for cows with LDPL (17.57%) compared with that recorded for cows subjected to SDPL (8.0%) and TDPL (6.13%). It was noticeable that elongation of the DPL resulted in significantly ( $p<0.05$ ) higher proportion of cows affected with CM (8.11%) compared with that observed for cows in SDPL (4.0%) or TDPL (4.8%) cow groups.

Our obtained results were harmonious with others (Gulay et al., 2003; Rastani et al., 2005) who proved that, DPL significantly impacted the incidence of mastitis with a tendency for lower SCC in SDPL (28–30 d). Moreover, these results came in agreement with Sawa et al. (2015) who recorded high proportion of CM in cows with LDPL (<90 days). Furthermore, it was supported by Pinedo et al. (2011) who stated that, LDP (143–250 days) increase the risk of SCM. Data of the present study were inconsistent with other investigators (Church et al., 2008; Watters et al., 2009; Safa et al., 2013) who failed to find significant effect of different DP lengths on the udder health in subsequent lactation. The inconsistent results concerning the effect of DPL on milk SCC may be due, to the different experimental approach (planned or retrospective studies) and the handling systems of lactating dairy cows (Soleimani et al., 2010). The results of the current study showed that, cows with SDPL had the best reproductive indices compared with cows in the other groups, a cows with SDPL showed shorter: calving to first estrus ( $83.67\pm 3.92$  d), calving to first service ( $107.28$ d), calving to conception ( $182.55$ d) intervals compared with values that recorded for both TDPL ( $98.32\pm 3.06$ d,  $133.39\pm 3.48$ d and  $233.32\pm 6.7$ d) and LDPL ( $155.17\pm 4.44$ d,  $193.23\pm 5.65$ d and  $281.51\pm 5.23$ d) respectively. These results are favored by Kuhn et al. (2006) who reported fewer DO during the subsequent lactation for cows with SDPs, and by Grummer (2007) who reported that SDPs resulted in improved postpartum fertility. Pezeshki et al. (2007) proved that SDPs associated with improved reproductive measures such as DO, CSI and NSPC. Watters et al. (2009) recorded a decrease in DO following a SDPs. Gümen et al. (2005)



reported a decrease of 24 and 20 days in DO following SDPs of 28 and 35 days respectively. However, Safa et al. (2013) concluded that, SDPs had no effect on days to first ovulation and number of follicles, also Pezeshki et al. (2008) observed no difference in DO for cows with a 49 or 28 DPL. Likewise, Annen et al. (2004) proved that reproductive indices did not significantly differ among cows assigned either to TDP (60-d), SDP (30-d) or continuous lactation. Further, DPL did not affect calving to service interval, CR at first service, CCI, or overall PR. However, Pezeshki et al. (2007) and Church et al. (2008) proved that, shortening the DP to less than 40 had an adverse effect on fertility in the subsequent lactation

It was obvious that, LDPL was associated with significantly lower first services CR ( $33.11\pm 2.91\%$ ), lower PR ( $50.39\pm 3.97\%$ ) within 150 DIM and higher NSPC ( $1.98\pm 0.37$ ) compared with values that recorded for either SDPL ( $38.19\pm 2.82\%$ ,  $58.32\pm 3.09\%$ ,  $1.71\pm 0.11$ ) or TDPL ( $43.16\pm 2.56\%$ ,  $63.35\pm 3.08$ ,  $1.58\pm 0.13$ ) respectively. These results are harmonic with Sawa et al. (2012) who stated that LDP was associated with reduced cow fertility in the next lactation, and supported by the findings of Pinedo et al. (2011) who reported that, LDPs were associated with increased CSI (89.4 d) and CCI (131.9 d) compared with that recorded for SDPs (83 d and 127.8 d respectively). Similarly, the NSPC increased with the length of previous DP from 1.58 in SDPL (31-52 d) to 2.44 in LDPL (143-250 d).

In the current investigation, a close association ( $p<0.05$ ) between udder health and reproductive efficiency was observed as the best reproductive values were recorded for cows with healthy udder compared with that recorded for cows with mastitic udder. Cows with CM required more days to come into detected estrus ( $126.74\pm 3.77$ d), more days to first service

( $172.2\pm 6.48$ d), more days to conceive after first AI ( $240.56\pm 4.68$ d) and more NSPC ( $2.69\pm 0.09$ ) compared with healthy udder cows. Moreover, clinically mastitic cows showed the lowest first service CR ( $18.91\pm 3.31\%$ ) and the lowest PR ( $32.54\pm 2.53\%$ ). Nearly the same reproductive trend was recorded for cows suffered from SCM. The obtained data favored by the results of earlier studies (Soto et al., 2003; Hansen et al., 2004) who declared a close relationship between mastitis and poor reproductive efficiency. Moreover, Lavon et al. (2011) reported that, approximately 14.3% decrease in CR among subclinical mastitic cows compared with control cows. A single threshold of SCC linear score at ( $\sim 300,000$  cells/mL) before or after insemination for SCM was associated with low CR (Pinedo et al., 2009). König et al. (2006) observed a low PR in cows showing increase of SCC  $>400,000$  cells/mL, but not in cows with mild increase of 150,000 to 400,000 cells/ml. Schrick et al. (2001) detected an increase in DO and NSPC only for CM, not SCM affected cows. Furthermore, Lavon et al. (2011) concluded that, mastitis is associated with a significant reduction in CR and the degree of the reduction is related: to mastitis type; clinical or subclinical, to the level of SCC elevation, and to its exact timing of elevation relative to insemination. Moreover, Kumar et al. (2017) concluded that, clinical mastitic cows had higher intervals: to first estrus, to first insemination, to conception and higher NSPC compared to clinically healthy cows.

In general, the results of this study revealed that, if mastitis occurred during the VWP, a significantly ( $p<0.05$ ) prolonged fertility interval parameters were observed, however, if mastitis occurred during the SP, a significantly ( $p<0.05$ ) lower CR, lower PR and higher NSPC were observed. The findings of this study and those reported by other researchers collectively indicate that the leftover effects of mastitis were

more significant when it occurred during the SP rather than during the VWP. Our results are coinciding with many other researchers. Ahmadzadeh et al. (2009) reported that cows experienced mastitis before the end of the VWP had greater NSPC and longer DO, suggesting that occurrence of CM before the first postcalving service may induce a negative impact on the reproductive efficiency. Lavon et al. (2011) also stated clearly that a CM diagnosed during the first 10 days before first postpartum insemination depresses fertility. In addition, Villa-Arcila et al. (2017) reported that, SCM in the first month after calving was associated with a longer calving to conception interval and a higher NSPC. Moreover, Fuenzalida et al. (2015) concluded that, cows experiencing SCM and CM during the SP had a reduced probability of pregnancy compared with healthy cows. The negative effects of CM on reproduction parameters were higher when CM occurred during the SP (Kumar et al., 2017). Others have established that, occurrence of intramammary infection around insemination is associated with greater reductions in the probability of pregnancy as compared with that occurs earlier or later (Lavon et al., 2011; Hudson et al., 2012). Furthermore, Maizon et al. (2004) stated that intramammary infection that occurred immediately postpartum resulted in: longer calving to first service; longer calving to conception intervals and higher NSPC. It has been reported that clinically mastitic cows had significantly higher days to first estrus, days to first insemination, days open, and NSPC compared to healthy udder cows (Nava-Trujillo et al., 2010; Kumar et al., 2017). CM and SCM are associated with an increase in: the number of open cows, the interval from calving to first service and the NSPC (Schrick et al., 2001; Santos et al., 2004; Hockett et al., 2005). High SCC resulted in longer intervals from calving to first service and to conception (Pinedo et al.,

2009), as well as in lower CR and lower PR (Santos et al., 2004). Others have revealed an impaired reproductive performance in cows with SCM (Gómez-Cifuentes et al., 2014). In fact, more abortion or early embryonic mortality was reported by several workers (Moore et al., 2005) when CM occurred between first postpartum insemination and conception. Kumar et al. (2017) stated that existence of CM after 62 days postpartum had more negative influence on fertility outcomes compared with that occurred during the first 4 weeks postpartum, which is in agreement with the findings reported by Nava-Trujillo et al. (2010). On the contrary, Huszenicza et al. (2005) found no impact of CM on the PR or the DO. Villa-Arcila et al. (2017) reported that, subclinical mastitis was not associated with calving to first service interval; however, the calving to conception interval (days open) was longer in cows with high SCC and also had a higher NSPC compared to healthy cows. Nava-Trujillo et al. (2010) proposed that negative energy balance (NEB), deficient dry matter intake, massive BCS loss, low blood glucose, low insulin and insulin like growth factor-I levels could be the potential explanations behind the extended calving-first estrus interval. Moreover, high milk yield may result in severe NEB, which is known to influence fertility (Wathes et al., 2007). Moreover, it had been accounted for that, elevated concentrations of NEFA and diminished concentrations of IGF-1 was related with NEB which can prompt decreased follicular development, debilitated estradiol amalgamation, and postponed ovulation (Wathes et al., 2007).

The precise mechanisms by which mastitis disturbs fertility not yet completely understood but could be attributed to the fact that mastitis induced release of cytokines, interleukins, and PGF2 $\alpha$  that may lead to failure in GnRH secretion (Schrick et al., 2001; Hockett et al.,

2005), disturb LH secretion and steroidogenesis, delay or hinder the preovulatory LH surge (Suzuki et al., 2001) resulting in either delayed or anovulation (Lavon et al., 2011) and consequently lowered fertility. Around insemination, mastitis may disturb maturation and fertilization of the oocyte (Soto et al., 2003). If mammary infection occurred around services, may obstruct CL formation, progesterone synthesis, uterine functions, and early embryonic progress (Mann and Lamming, 2001; Spencer et al., 2004). Another method through which mastitis reduce the reproductive performance may be explained by the effect of inflammatory mediators on the pituitary and gonadal hormones (Hertl et al., 2010; Hertl et al., 2014). These mediators may initiate and augment febrile reactions, inhibiting the release of gonadotropins requisited for follicular growth and steroidogenesis (Huszenicza et al., 2004; Pate et al., 2010), as granulosa cells have receptor sites for interleukins, chemokines and TNF $\alpha$  that interfere with the synthesis of estrogens (Herath et al., 2007; Bromfield and Sheldon, 2011). It has been reported that, cows with SCM showed substandard estrogen levels, lowering the positive feedback effect of estrogen leading to either delayed or anovulation (Lavon et al., 2011). Furthermore, the presence of chemical mediators that would affect the viability of the embryo might explain the detrimental effect of mastitis on the fertility potentials (Soto et al., 2003; Huszenicza et al., 2004). Similarly, bacterial endotoxins may induce the release of prostaglandins and cortisol, which would have an adverse effect on the development and quality of the oocyte and the CL (Schrick et al., 2001; Hockett et al., 2005). Additionally, CM may induce earl embryonic death which may occur due to the action of pro-inflammatory cytokines (Hansen et al., 2004). Furthermore, delayed days to first estrus in CM diseased cows might be due to altered

hypothalamic–pituitary hormonal axis (Hansen et al., 2004). As well, the direct connection between SCM and endometritis has been suggested by Bacha and Regassa (2010). This would confirm the effect of mastitis on reproduction and would indicate that this effect is mediated by multiple mechanisms, not only at the pituitary level but also at the gonadal level.

## **5. Conclusions**

A close connection among the dry period length, udder health status and reproductive performance of Holstein-Frisian cows were observed, with the best results were obtained for cows with traditional and short dry period lengths as well as cows with healthy udder. The occurrence of mammary infection during the voluntary waiting period (i. e. before the first postpartum insemination) increased the calving to first estrus and first service intervals, while its occurrence during the service period, prolonged the calving to conception interval, and reduced the conception and pregnancy rates. It is recommended that, the dry period should be between 30 and 60 days and restrict control measures must be taken in consideration to combat both clinical and subclinical mastitis as early as possible especially during the service period to obtain good dairy herd fertility results.

## **Conflict of interest**

The authors declare that they have no conflict of interests.

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