Factors associated with abnormal resumption of ovarian activity after calving of Holstein-Friesian cows reared in northern Algeria

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Abstract

The objectives of the present study were to identify the factors influencing the resumption of luteal activity, to describe the incidence of different categories of postpartum ovarian activity and to investigate the relationships among parity, calving season, body condition score (BCS) at calving, BCS loss from calving to 30 days of lactation (DIM), and abnormal resumption of ovarian cycles postpartum of 84 Holstein-Friesian cows in northern Algeria. Blood samples were collected on days 30, 42 and 54 after calving and serum progesterone concentrations were measured by enzyme immunoassay to determine the luteal activity (≥ 1ng/mL) and different types of luteal dysfunctions in cows. The luteal activity was estimated at 39% at 30 DIM, 57% at 42 DIM and 69% at 54 DIM. The onset of luteal activity was influenced by milk production at 30 DIM. Cows producing less than 3000 kg of milk per year were 1.43 times more likely to have early luteal activity. However, no significant influence was reported at 42 and 54 DIM. 38%, of cows had normal resumption and 62%, had abnormal cyclicity, where 31 had delayed first ovulation and 31%, presented prolonged luteal phase. Using multiple ordinary logistic regressions, significant associations between parity, calving season and abnormal ovarian cyclicity were identified. Primiparous cows were 6.6 times more likely to have delayed first ovulation as multiparous cows (OR=7.60, $p<0.05$). Calving in cold season increased significantly the risk for delayed of first ovulation (OR= 12.68, $p<0.01$) and prolonged luteal phase (OR=8.85, $p<0.01$). In conclusion, more than half of the cows presented abnormal resumption of ovarian cyclicity which was influenced by parity and calving season.

Keywords: *calving season, dairy cow, ovarian cyclicity, parity, postpartum, reproduction*

Introduction

One of the crucial events to regain the maximum breeding potential of postpartum dairy cows is the early resumption of ovarian cyclicity. The aim is to achieve calving to first service (waiting period) and conception intervals (open days) of 55 and 85 days respectively, so that calving to calving intervals are maintained at 365 days. In confinement herds Northern Algeria, subsequent reproductive performance in lactating cows is declined, as reflected by prolonged open days and calving to calving intervals (Madani et al 2008). Thus, the increased frequency of abnormal resumption of ovarian activity after calving such as delayed first ovulation and prolonged luteal phase is associated with degradation of reproductive performance (Petersson et al 2006).The causes of this ovarian dysfunctions are multifactorial involving parity, season of calving, severes body condition score (BCS) loss, negative energy balance, milk production yield and clinical diseases (Opsomer et al 2000; Meikle et al 2004; Lucy 2008 and Kacar et Kaya 2014). The aims of the present study were to identify the factors influencing the resumption of luteal activity, to describe the incidence of different categories of postpartum ovarian activity and to investigate the relationships among parity, calving season, BCS at calving, BCS loss from calving to 30 days after calving, and abnormal resumption of cyclicity after calving in Holstein-Friesian cows in Northern Algeria.

Materials and methods

Animals and herd

The study was conducted on 84 Holstein-Friesian cows (ranged from 2 to 11 years) reared in the Technical Institute of Livestock Breeding, in Baba Ali-Algiers (Longitude 3.067° and Latitude 36.667°). The area has Mediterranean climate with two distinct seasons, a warm summer (with peak temperatures reaching 33°C) and cold winter (with a minimum temperature below freezing) and a pluviometry comprised between 600 and 800 mm/year. Cows were kept in the tie-stall barns and fed forage (sorghum, berseem, corn silage and alfalfa hay), concentrate (corn, barley, wheat bran) and commercial concentrate for lactation with trace minerals and vitamins. Cows were machine-milked twice daily and the average 305 days milk production was 3414±1186 kg (ranged from 1950 to 6600 kg).

Blood sampling and progesterone assay

Blood samples were collected after morning milking from the coccygeal vein into vacutainer tube without anticoagulant. Samples were centrifuged at 1,500 x g for 10 minutes. Separated serum samples were collected and stored at -20°C until assay. The luteal activity was determined by serum progesterone concentrations on days 30 (d30), d42 and d54 after parturition. Progesterone (P4) concentrations were quantified by enzyme immunoassay (ST AIA-PACK PROG, Tosoh Co, Ltd, Japan). The minimal detectable concentration (sensitivity) was estimated to be 0.1ng/mL. Intra-assay and inter-assay coefficients of variation were 9.9 and 11.3 %, respectively. A functional *corpus luteum* (CL) was considered to be present if serum P4 concentrations were ≥ 1ng/mL (Stevenson et al 2006). Ovulation was considered to have occurred 5 days before the first rise day of luteal activity. Cows were classified into the following three groups, based on serum P4 profiles (Shrestha et al 2004).

- Normal resumption (NR): ovulation occurred ≤ 45 days after calving, followed by regular ovarian cycles.

- Delayed first ovulation (DFO): first ovulation did not occur until > 45 days after calving.

- Prolonged luteal phase (PLP): ovulation occurred ≤ 45 days postpartum, but more ovarian cycles had luteal activity for > 20 days.

Clinical reproductive examination of cows

In order to control uterine involution and ovarian activity of cows, rectal palpations were conducted during blood sampling.

Body condition score assessment

Body Condition Score was measured three times: at calving (BCS0), on day 30 (BCS30) and 60 (BCS60) after calving, on a scale from 1 (very thin) to 5 (fat) with 0.5 increments. All BCS were assigned by the same operator using the visual technique developed by Edmonson et al (1989).

Milk yield

The estimated 305 days milk yields of individual cows (n=66) were obtained from the Technical Institute of Livestock Breeding, Algiers.

Statistical analysis

Prior to statistical analysis, observations were checked for unlikely values; no data were excluded for this reason. Analyses were performed with SAS Software (version 9.1.3; SAS Institute Inc, Cary, NC, USA). Descriptive statistics for qualitative and quantitative variables under study were computed. The incidences of the different types of ovarian cycles postpartum during the pre-service period across cow-level factors were expressed as percentages. The GENMOD procedure of SAS was used for analyses the effect of parity (multiparous *vs*. primiparous), season calving (cold *vs*. warm), BCS, BCS loss and total milk yield on luteal activity (at 30, 42 and 54 DIM). The BCS at calving was categorized as: high (≥

4.0), moderate (3.0-3.5) and low (≤ 2.5), BCS loss from calving to 30 DIM was classified into two categories (≥1, < 1); season calving was grouped as cold (October to March) and warm (April to September). Total milk yield was also dichotomized into three groups (< 3000, 3000-4500 and >4500 kg/year). Thus, effects of these covariates on abnormal resumption (DFO and PLP) of cyclicity compared with normal resumption (NR) after calving in Holstein-Friesian cows were examined by using multiple ordinary logistic regressions of SAS. Odds Ratio (OR) and 95% confidence intervals (95%-CI) were determined.

Results

Characteristics of dairy cows

61% of cows *w*ere primiparous (2.6±0.5 years), calved generally in the cold season (57%). 56% of cows had an average BCS at calving of 3.3±0.3. For most of them, the loss of BCS between the calving and 30 DIM was estimated at 0.5 point (<1 point). For more than half of cows (53%), the average 305 days milk yields remained low (2503±228 kg per year) (Table 1).

Table 1. Descriptive analysis of qualitative and /or quantitative variables

Descriptive variables	Definition of modalities	n (Mean±SD)	Percent
Parity	Primiparous	51 (2.6±0.5)	61
	Multiparous	33 (6.1±2.01)	39
Calving season	Cold	48	57
	Warm	36	43
BCS at Calving	Low (2-2.5)	24 (2.4±0.2)	29
	Moderate (3-3.5)	47 (3.3±0.3)	56
	High (4-4.5)	13 (4.2±0.2)	15
BCS at 30 DIM	Low (2-2.5)	46 (2.3±0.3)	55
	Moderate (3-3.5)	33 (3.2±0.2)	39
	High (4-4.5)	5 (4)	6
BCS at 60 DIM	Low (2-2.5)	34 (2.4±0.2)	41
	Moderate (3-3.5)	44 (3.1±0.2)	52
	High (4-4.5)	6 (4.2±0.3)	7
BCS loss	<1	78	93
	≥1	6	7
Total Milk Yield (kg/year)	< 3000	35 (2503±228)	53
	3000-4500	16 (3691±508)	24
	> 4500	15 (5244±613)	23

n:number of cows SD: Standard Deviation

Concentration of progesterone and ovarian activity

At 30, 42 and 54 DIM, the luteal activity was estimated at 39%, 57% and 69%, respectively. Concerning the ovarian status of cows, out of 84, only 32 cows (38%)

had normal resumption of ovarian cycles postpartum, where 52 cows (62%) had an abnormal ovarian cycle with 31% of DFO and 31% of PLP (Table2).

Table 2. Estimation of luteal activity and incidence of different categories of postpartum ovarian activity

	Number of cows	Percent
Estimation of postpartum luteal activities (≥ 1ng/mL)		
30 DIM	33/84	39
42 DIM	48/84	57
54 DIM	58/84	69
Categories of ovarian activities		
Normal Resumption	32/84	38
Delay of First Ovulation	26/84	31
Prolonged Luteal Phase	26/84	31

Relationships between parity, calving season, BCS at calving, BCS loss and total milk yield on luteal activity

At 30 DIM, cows producing less than 3000 kg per year were more likely to have early luteal activity (OR =1.43, $p<0.05$) compared with those producing more than 4500 kg/year. However, this difference was not significant for cows producing between 3000-4500 kg per year. Also, there were no significant effects ($p>0.05$) of parity, calving season, BCS at calving, BCS loss and total milk yield on the luteal activity at 42 and 54 DIM (Table 3).

Table 3. Effects of parity, season, BCS0, total milk yield and BCS loss on the luteal activity at 30, 42 and 54 DIM.

Factors	Class (n)	30 DIM	42 DIM	54 DIM
		Odds Ratio (95%-CI)		
Parity	Multiparous (33)		Ref.	
	Primiparous (51)	0.80 (0.62-1.03) (17/51)	1.20 (0.62-2.11) (27/51)	1.34 (0.79-2.25) (31/51)
BCS0	Moderate (47)		Ref.	
	High (13)	1.04 (0.73-1.49) (8/13)	0.96 (0.41-2.00) (8/13)	1.20 (0.58-2.31) (11/13)
	Low (24)	0.81 (0.60-1.10) (8/24)	0.65 (0.30-1.28) (8/24)	0.83 (0.43-0.42) (14/24)
BCS Loss	< 1 (78)		Ref.	
	≥ 1 (6)	0.78 (0.57-1.08) (2/6)	0.86 (0.21-2.36) (4/6)	0.96 (0.29-0.29) (4/6)
Calving Season	Warm (36)		Ref.	
	Cold (48)	1.08 (0.84-1.38) (20/48)	0.88 (0.50-1.57) (26/48)	0.86 (0.51-1.45) (31/48)
Total Milk	>4500 (15)		Ref.	

Yield kg/year	<3000 (35)	**1.43*** (1.05-1.94) (16/35)	1.27 (0.57-3.22) (22/35)	1.36 (0.65-3.22) (26/35)
	3000-4500 (16)	1.36 (0.97-1.89) (7/16)	1.37 (0.51-3.85) (10/16)	1.20 (0.46-3.21) (7/16)

*(n): number of cows *p <0.05 CI : confidence intervals*

Incidence of different types post-partum cycles during the pre-service period across parity, season calving, BCS at calving and BCS loss groups

Results presented in Table 4 showed that the late resumption was more observed in primiparous cows than multiparous ones (39 *vs* 18%). Calving in cold season presented DFO and PLP compared with warm season (35 *vs* 25%; 36 *vs* 22%, respectively). 42% of cows that had calved with low BCS showed DFO compared with moderate BCS at calving (30%). The proportions of cows having lost half a point of BCS between calving-30 DIM were 37, 31 and 32% in normal resumption, DFO and PLP, respectively.

Table 4. Incidence of different types of ovarian cycles postpartum during the pre-service period across parity, calving season, BCS at calving and BCS loss groups.

Factor	Class (n)	DFO (%)	PLP (%)	NR (%)
Parity	Multiparous(33)	6 (18)	13 (39)	14 (43)
	Primiparous(51)	20 (39)	13 (26)	18 (35)
Calving season	Cold (48)	17 (35)	18 (38)	13 (27)
	Warm (36)	9 (25)	8 (22)	19 (53)
BCS0	High (13)	2 (15)	3 (23)	8 (62)
	Low (24)	10 (42)	6 (25)	8 (33)
	Moderate (47)	14 (30)	17 (36)	16 (34)
BCS loss	<1 (78)	24 (31)	25 (32)	29 (37)
	≥1 (6)	2 (23)	1 (17)	3 (50)

n : number of cows DFO: Delay First Ovulation, PLP: Prolonged Luteal Phase, NR: Normal Resumption

Risk factors associated with abnormal resumption of ovarian activity

In the present study, two significant risk factors associated with abnormal resumption of ovarian activity were observed (Table 5). Primiparous cows were more likely to have DFO (more than 42DIM) by 6.6 times (OR=7.60, $p<0.05$) than multiparous cows. However, multiparous cows were equally likely (OR=1.12, $p>0.05$) to have PLP than primiparous cows.

Moreover, calving in cold season increased the risk for DFO by 11.6 times (OR=12.68, $p<0.05$) and PLP by 7.8 times (OR=8.84, $p< 0.05$) compared with warm season. Moreover, the regression model did not reveal significant effects ($p>0.05$) of BCS at calving and BCS loss on abnormal resumption of ovarian activity after calving in Holstein-Friesian cows.

Table 5. Effects of parity, calving season, BCS0, and BCS loss on abnormal resumption of ovarian activity after calving in Holstein cows.

Abnormal resumption	Factor	Class	Odds Ratio	95%-CI
DFO	Parity	Multiparous	Ref.	
		Primiparous	**7.60***	1.02-56.25
	Calving season	Warm	Ref.	
		Cold	**12.68****	2.23-71.84
	BCS0	Moderate	Ref.	
		High	0.86	0.02-0.96
		Low	1.95	0.40-9.50
	BCS loss	≥1	Ref.	
		<1	1.74	0.13 -22.63
PLP	Parity	Primiparous	Ref.	
		Multiparous	1.12	0.24-5.28
	Calving season	Warm	Ref.	
		Cold	**8.84****	1.77-43.97
	BCS0	Moderate	Ref.	
		Low	0.96	0.20-4.52
		High	0.14	0.02-1.16
	BCS loss	≥1	Ref.	
		<1	0.44	0.02-6.75

**p<0.05; **p<0.01. CI: Confidence Intervals DFO: Delay First Ovulation, PLP: Prolonged Luteal Phase*

Discussion

Attaining pregnancy of dairy cows within 85 days after parturition requires an early resumption of ovarian cyclicity and onset of estrus postpartum which consequently allows high reproductive potential of dairy cows (Gautam et al 2010; Kocila et al 2013). For example, the mean interval from the calving to resumption of ovarian activity was approximately 25 to 40 days postpartum with a typical range of 17 to 50 days (Butler et Smith 1989; Staples et al 1990; Akhtar et al 2015). According to Tamadon et al (2011), 72% of cows have resumed luteal activity earlier than 45 days after calving. However, in our study, only 39% of the cows (33/84) resumed their ovarian activity at 30 days after parturition, relatively lower than that reported by Disenhaus et al (2008) (52%) and Ghanem (2016) (47.5%), but not far from reported by Galvao et al (2010) and Kalem et al (2017). The onset of luteal activity at 30DIM can be explained, in the present experiment, by low level of milk production (<3000 kg/year) (OR=1.43, $p<0.05$) and consequently of a less serious negative energy balance. According to the literature, the risk for delayed cyclicity was higher when milk production was high (Grimard et Disenhaus 2005; Peter et al 2009). It has been suggested that higher milk production reduces the likelihood that the first dominant follicle will ovulate (Beam et Butler 1999). On the other hand, an earlier study has found no relationship between milk production and reproduction up to 5900 kg/year (Buckley et al 2003).

Abnormal patterns of resumption of cyclicity have often been identified in Holstein-Friesian cows in numerous countries (Grimard et Disenhaus 2005; Crowe 2008). In the current study, serum levels of progesterone showed a higher incidence of abnormal ovarian cycles compared with normal ovarian cycles (62% *vs* 38%). Several studies have revealed similar rates, (55% *vs* 45% n=37) (Taylor et al 2003); (63% *vs* 37% n=54) (Shrestha et al 2004); (55.5% *vs* 44.5% n=110) (Shrestha et al 2005); (57.1% *vs* 42.9% n=21) (Hommeida et al 2005) and (52.5% vs 47.5% n=61) (Ghanem 2016). However, more recent studies have not confirmed these results. Indeed, a higher frequency of normal resumption of ovarian activity postpartum was reported by Ledoux et al (2011) (56.5% *vs* 43.5% n=239) and Tamadon et al (2011) (71.8% *vs* 28.2% n=71).

Delayed first ovulation and PLP were the most frequently observed problems in this study which concords with previous experiments (Petersson et al 2006; Ledoux et al 2011). Incidence of DFO (31%) was higher than observed by Shrestha et al (2005) (24.1%), Shrestha et al (2004) (12.7%) and Ledoux et al (2011) (16.3%) but similar to that reported by Hommeida et al (2005) (33.3%). In the current study, parity and calving season were significant ($p<0.05$) risk factors for DFO. Primiparous cows had 6.6 times more risk of suffering from DFO compared with multiparous cows. This relationship was in accordance with reports that primiparous cows had more days to first insemination than multiparous cows (115 *vs* 105 days, $p<0.05$) (Folnožić et al 2016). The later ovulation observed in primiparous cows can be explained, in this study, by several hypotheses as metabolic problems, calving problem and occurrence of puerperal disturbances (metritis), as previously reported by Vercouteren et al (2015). Calving in the cold season had 11.7 times more risk of suffering from DFO compared with calving in warm season. This result was also reported in a field study conducted by Opsomer et al (2000) who reported that the most unfavorable breeding season being winter, knowing that 38% of cows calving in the winter did not resume ovarian activity during the first 50 days after calving. Our results may be explained by the fact that cows were housed in stables and therefore there were feeding and locomotion problems during winter (Opsomer et al (2000). Furthermore, calving in a stable was a risk factor for abnormal ovarian function when compared with calving in pasture.

However, no significant differences *(p>0.05)* were observed in this study, between BCS at calving, BCS loss from calving to 30 DIM and DFO.

In our experiment, incidence of PLP (31%) was higher than those observed by Ledoux et al (2011) (18.4%) and Ghanem (2016) (21.3%) but similar to that reported by Hommeida et al (2005) (28.3%) and Shrestha et al (2005) (33.6%). Calving season was the most risk factor for developing PLP. Cows calving in the cold season had 7.8 times more risk of suffering from PLP compared with cows calving in warm season. This risk factor could be associated with retained placenta, abnormal vaginal discharge, metritis, as previously reported (Opsomer et al 2000; Taylor et al 2003; Shrestha et al 2004; Kacar et kaya 2014). In addition, these puerperal pathologies may compromise the uterine ability to produce PGF2a (Baranski et al 2013). This was likely to be one of the factors predisposed to PLP in our experiment. Finally, the logistic regression model showed no significant effects*(p>0.05)* between PLP and parity, BCS at calving and BCS loss from calving to 30 DIM.

Conclusion

- In conclusion, high incidence of abnormal ovarian cycles was observed. Delayed first ovulation and prolonged luteal phase were the main abnormal patterns of ovarian cyclicity, wich were influenced by the parity and the calving season.
- Primiparous cows were a significant risk factor for DFO. Calving in cold season was more risk factor of suffering from DFO and PLP.
- Housing in tie-stall barns, energy deficiency, physical inactivity and clinical disorders, observed during winter calving, were considered the main causes of reproductive failure.

Conflict of interests statement

The authors declare that there is no conflict of interests regarding the publication of this article.

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