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Effect of lameness on follicular dynamics in crossbred cows

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ABSTRACT

Lameness impedes reproduction in dairy cows. It is not clear whether follicular dynamics is also involved. Ovarian ultrasonography was performed once daily for one to two interovulatory intervals in the cyclic cows that were either normal (N) or lame (L). A 2-wave pattern of follicular growth predominated and was therefore utilized to compare the follicular dynamics during six and 13 interovulatory intervals, respectively, in six N and 10 L cows. At wave emergence, the follicular characteristics were similar between the N and the L cows. Subsequently, the L cows compared to the N cows exhibited an increased number of small ($P<0.05$), medium ($P<0.01$) and large ($P<0.05$) sized-follicles during the anovulatory and ovulatory follicular waves; delayed selection of the dominant follicle in the anovulatory (day 3.6 ± 0.2 versus day 2.8 ± 0.1; $P<0.05$) as well as the ovulatory wave (day 5.6 ± 0.2 versus day 2.8 ± 0.1; $P<0.01$) and longer persistence of the ovulatory follicle (10.9 versus 9.4 days; $P<0.05$). It was concluded that the follicular composition and characteristics were disturbed in the lame cycling cows.

Key words: follicular dynamics, lameness, crossbred cows

Introduction

Crossbreeding initiated in several tropical countries has met with limited success (DESHMUKH and KAIKINI, 1999) primarily due to the increased vulnerability of the crossbred cows to certain diseases (LOSSOS, 1986) including lameness. Incidence of lameness is increasing in Indian tropical crossbreds (SOOD, 2005) and European (ESPEJO et al., 2006) cattle. Lame cows have a significantly - delayed calving to conception interval,

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higher number of services per conception and reduced conception rates (HERNANDEZ et al., 2005; SOOD, 2005). For normal conception, a sequence of coordinated follicular changes must culminate in ovulation of a healthy ovarian follicle (RIZO et al., 2008). Information on ovarian follicular dynamics is lacking in lame crossbred cows and was therefore investigated.

Materials and methods

The synopsis of this study was approved by the University's "Animal Ethics and Welfare Committee", an official organ of "The National Animal Ethics Committee". The lame cows in this study were neither created nor kept lame for the sake of the study.

Animals and management. The study was conducted on a total of 19 normally calved crossbred dairy cows (Holstein Friesian x Sahiwal; exotic inheritance of 50.0 to 62.5%) in the breeding period. The cows belonged to the Dairy Farm of Punjab Agricultural University, Ludhiana, India; were housed loose and fed with *ad libitum* seasonal green fodder, wheat straw, silage and an adequate quantity of concentrate ration (ANONYM., 2001).

Scoring of lameness and selection of cows. Lameness scoring was always done as per the method of WELLS et al. (1993) by the first author observing the cows walking on a 20 m firm walkway following evening milking.

Eleven cows with moderate lameness (score of 2; characterised by moderate and consistent gait asymmetry or symmetric gait abnormality, but able to walk without continuous stimulation) due to interdigital wounds (n = 9) or painful swelling with a wound on the hock region (n = 2) were selected. All the lame cows received a five day course of 2.5 g (b.i.d) Streptopenicillin [Munomycin®, India] and 10 mL (b.i.d) of Meloxicam [Melonex™, India] by intramuscular route. The lesions causing lameness were also treated locally by cleaning with warm 0.9% normal saline solution followed by application of self prepared Bismuth Iodoform Paste [BIPP; Bismuth subnitras 1 part, Iodoform 2 parts and Liquid paraffin q.s.]. Another eight non-lame (N) herd mates (score of 0) were also included for control observations.

Each of the selected N and L cows was followed weekly for the presence and severity of lameness during the course of the study.

The reproductive status of the cows was ascertained from history, ultrasonography and transrectal palpation of the genitalia. All the cows remained cyclic throughout the study. At the start of the experiment, lactation number, postpartum stage, body weight, body condition score (BCS, 0.25 unit increments in a scale of 1 to 5) (EDMONSON et al., 1989) and milk yield were recorded.

Follicular dynamics. Investigations on follicular dynamics in the lame cows began 5 to 10 days after the systemic treatment. During major part of the study, lame cows remained under local treatment. All the selected N and L cows were subjected once daily to ovarian follicular and luteal characterization through ultrasonography (PIERSON and GINTHER, 1988) using a 5 MHz linear array rectal transducer in a B-mode ultrasound scanner (Concept MCV, Dynamic Imaging, Scotland, U.K.). The observations began from the day of estrus and lasted for 19 to 46 days through one to two interovulatory interval(s) (IOI). The day of ovulation was defined as day 0 and constituted the beginning of an IOI.

During each examination, the number, size and location of ovarian follicles and C.L were recorded. The components [dominant follicle(s) DF(s); subordinate follicle(s) SF(s)] and characteristics [growth, static and regressing phases and persistence of the DFs] of follicular waves were determined as per GINTHER et al. (1989a,b) with slight modifications in categorizing the follicles on the basis of their size. Due to smaller maximum size, the follicles of >3≤ 5, >5≤7 and >7 mm size were considered as small, medium and large, respectively. The DF was at least 7 mm and exceeded the diameter of the second largest follicle (hereafter mentioned as largest SF) in the wave. Selection of a DF was as defined by GINTHER et al. (1996); the average value in the N and L cows has been presented. The nearest whole mean value pertaining to length of IOIs and day of wave emergence were considered for depicting the number of follicles of different sizes and DF characteristics on different days of the estrous cycle. The estrous cycles with one, two or three waves were classified as 1-, 2- or 3- wave cycles, respectively. The size of CL was recorded as cross sectional trace area (mm^2).

Blood samples. Heparinized blood samples were collected weekly from each cow through direct jugular venupuncture and placed on ice. Within 1h, plasma was separated by centrifugation at 2500 g for 15 min and stored at -20 ^oC. Plasma concentrations of progesterone (KAMBOJ and PRAKASH, 1993) and non-esterified fatty acids (NEFA) (FOLCH et al., 1957) were determined. The blood samples were always collected between 1200 to 1300 h, i.e 3 to 5 h after morning feeding.

Data analysis. The average body weight, BCS and milk yield between the N and the L cows was compared using Student's *t*-test.

Sizes of the anovulatory DFs for the first seven days and OFs for the first ten days and the number of follicles of different sizes were analysed in the N and the L cows by the Statistical Analysis System general linear models univariate analysis of variance of repeated measures with degrees of freedom modified by Green house-Geyser epsilon (SAS / STAT). The statistical model included the effects of treatment (N vs. L cows), the cow (within treatment), day of estrous cycle and treatment-by-day interaction. Data were analysed for the entire estrous cycle and separately for the anovulatory and ovulatory

waves. Differences in the average diameter and other characteristics of DFs, SFs, size of CL on daily basis and plasma NEFA concentrations were compared between the N and L groups using Student's *t*-test.

The plasma progesterone concentrations were used to confirm estrous cyclicity and not analysed statistically.

Results

The moderate degree of lameness at the initiation of the study decreased to the mild form (score of 1; characterized by mild gait asymmetry and/or restriction in free movement) by the end of study in all the L cows.

The lactation number, postpartum stage, BCS, body weight and daily milk yield ranged from 3 to 4, 69 to 75 days, 3.0 to 3.3, 387 to 435 kg and 9.5to 12.0 l/d, respectively and did not differ ($P>0.05$) between the N and the L cows.

The plasma progesterone concentrations fluctuated between 0.3 and 2.3 ng/mL and corroborated to different phases of the estrous cycle in the N and the L cows.

Follicular dynamics and CL changes in the N and the L cows. Following estrus detection, a total of 23 IOIs, nine in eight N and 14 in 11 L cows, respectively, were monitored through one or two consecutive IOIs. Two-wave follicular activity predominated during six IOIs (66.7%) in six N and 13 IOIs (92.9%) in 10 L cows and was utilized to elaborate the effects of lameness. The remaining cows had either 1-wave (7.1%, one L cow) or 3-wave (33.3%, two N cows) follicular activity (data not presented). The one N and three L cows examined for two consecutive IOIs repeated 3- and 2- wave pattern, respectively.

The average duration of IOI in the N and the L cows was 20.5 ± 0.2 and 21.6 ± 0.4 days that was normalized to 21 and 22 days, respectively. Similarly, the emergence of an anovulatory wave on day 0.5 ± 0.1 in the N and day 0.2 ± 0.06 in the L cows was assigned to day 0; and the ovulatory wave on day 11.2 ± 0.3 in the N and day 10.6 ± 0.4 in the L cows was assigned to day 11. The various follicular characteristics at the emergence of anovulatory and ovulatory waves were similar in the N and the L cows, although differences in the other wave characteristics were recorded (Table 1). There were a comparable number of small, medium and large - sized follicles at the beginning of the anovulatory wave. However, with the progression in wave, lameness altered the number of follicles of different sizes (Fig. 1). A treatment-by-day-interaction during the estrous cycle was detected for the number of small ($P<0.05$, lower panel), medium ($P<0.01$, middle panel) and large-sized ($P<0.10$, upper panel) follicles. The greatest disparity between the N and L cows was recorded for the number of medium-sized follicles. More of the medium-sized follicles were detected from day 4 to 11 in the L cows and the distinct decline observed in their number between day 4 to 7 in the N cows was much

slower in the L cows (P<0.01, middle panel Fig. 1). Compared to the anovulatory wave, there were even more medium-sized follicles during the ovulatory wave between day 15 to 20 in the L than the N cows (P<0.01, middle panel Fig. 1).

The average size of the anovulatory DFs and OFs on different days of estrous cycles was similar between the N and L cows. However, size of OFs in the L cows tended to differ from the N cows (treatment-by-day interaction, P = 0.10) (Fig. 2)

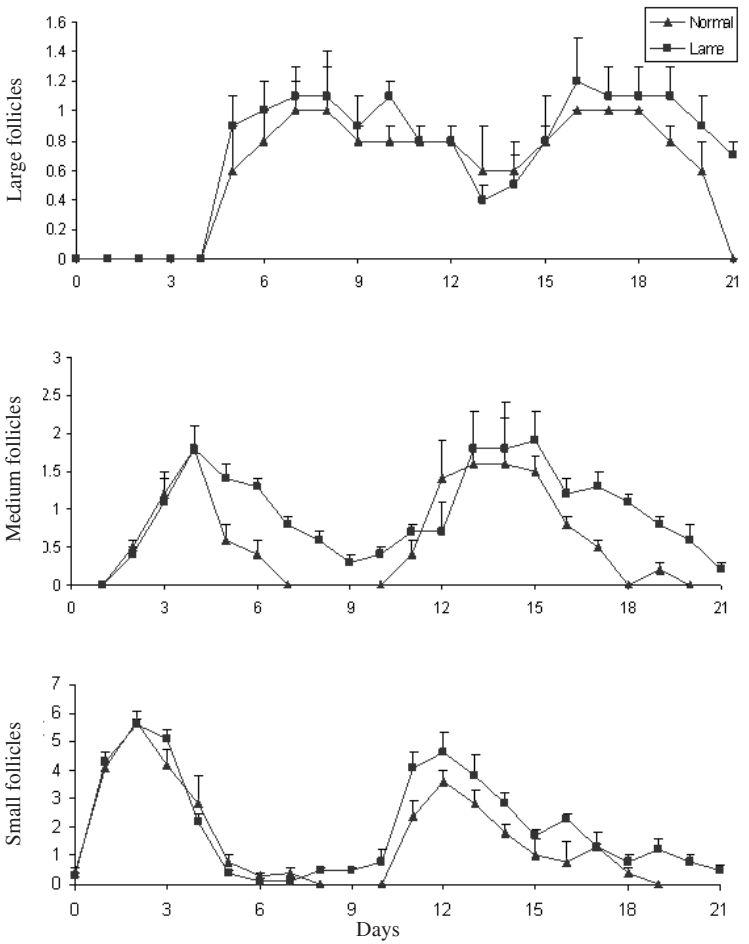

Fig. 1. Number of small (>3≤5 mm), medium (>5≤7 mm) and large (>7mm) follicles during estrous cycles with 2-wave follicular activity in normal (n = 6; interovulatory intervals = 6, solid triangles) and lame (n = 10; interovulatory intervals = 13, solid squares) crossbred cows

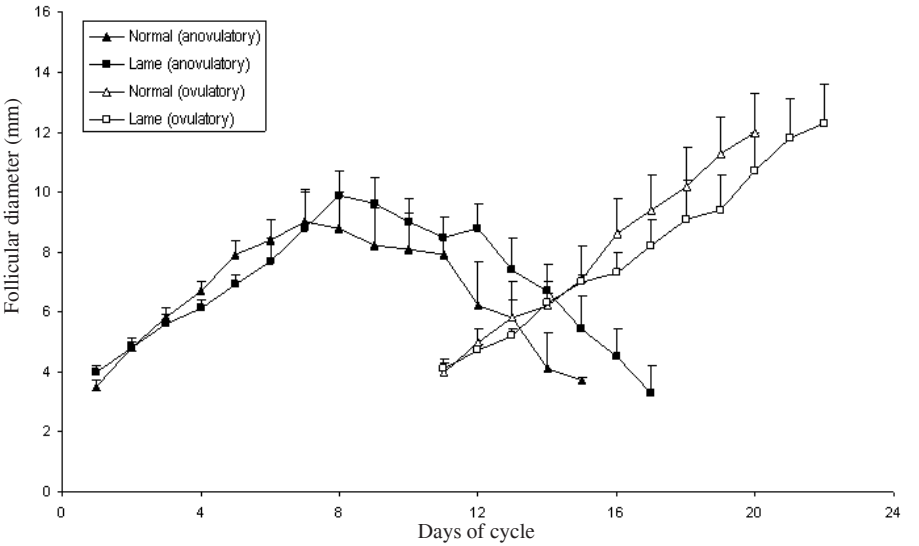

Fig. 2. Growth patterns of the dominant follicles during anovulatory and ovulatory waves during estrous cycles with 2-wave follicular activity in normal (n = 6; interovulatory intervals = 6, solid triangles, open triangles) and lame (n = 10; interovulatory intervals = 13, solid squares, open squares) crossbred cows

Selection of both, anovulatory DF and OF, occurred on day 2.8 ± 0.1 in the N cows, whereas anovulatory DF and the OF were selected on day 3.6 ± 0.2 ($P<0.05$) and day 5.6 ± 0.2 ($P<0.01$), respectively, in the L cows. There was no difference ($P>0.05$) in the diameter of the DF and the associated largest SF on the day of selection for anovulatory and ovulatory waves between the N and the L cows (data not presented).

The OF persisted for a longer duration in the L (six of the thirteen OF persisted for 12.0 ± 0.2 days) than the N cows. Furthermore, the largest SF in the ovulatory wave also persisted longer ($P<0.05$) in the L than the N cows (Table 1).

The CL attained a maximum size of 68.2 ± 1.9 mm^2 and 70.2 ± 2.8 mm^2 on day 6.9 ± 1.3 and day 7.1 ± 1.6, respectively, in the N and the L cows and did not differ in size on any day of the estrous cycle between the two groups.

The average concentrations of NEFA ranged from 89.4 ± 2.6 to 93.3 ± 2.4 mg/L and did not differ ($P>0.05$) between the N and the L cows.

Table 1. Certain characteristics of wave emergence, dominant and largest subordinate follicle during anovulatory and ovulatory waves in normal (N) and lame (L) crossbred cows exhibiting 2-wave follicular growth

Characteristic*	Anovulatory wave		Ovulatory wave	
	N	L	N	L
Wave emergence				
Day of first detection	0.5 ± 0.1	0.2 ± 0.06	11.2 ± 0.3	10.6 ± 0.4
Number of detected follicles	4.1 ± 0.3	4.3 ± 0.4	4.2 ± 0.5	3.9 ± 0.3
Dominant follicle				
Growing phase				
Beginning day	0.5 ± 0.2	0.2 ± 0.06	11.2 ± 0.3	10.6 ± 0.3
Diameter (mm)	3.5 ± 0.2	4.0 ± 0.2	4.0 ± 0.3	4.1 ± 0.3
Linear growth rate (mm/day)	1.1 ± 0.08	1.0 ± 0.1	0.9 ± 0.04	0.8 ± 0.3
Ending day	6.3 ± 0.4	6.5 ± 0.6	20.5 ± 0.2	21.7 ± 0.6
Diameter (mm)	9.5 ± 0.8	10.5 ± 0.7	12.6 ± 0.2	12.3 ± 0.5
Static phase (days)	4.8 ± 0.8	5.1 ± 0.6	-	-
Regressing phase			-	-
Beginning day	10.9 ± 1.4	11.2 ± 0.9	-	-
Linear regression rate (mm/day)	-1.1 ± 0.06	-1.0 ± 0.1	-	-
Ending day	14.3 ± 2.2	15.8 ± 1.1	-	-
Diameter (mm)	3.4 ± 0.1	3.7 ± 0.3	-	-
Persistence (days)	13.7 ± 1.2	15.6 ± 0.8	9.5 ± 0.2[a]	11.2 ± 0.5[b]
Largest subordinate follicle				
Detection day	0.7 ± 0.1	0.4 ± 0.2	11.9 ± 0.4	11.2 ± 0.3
Maximum diameter (mm)	4.8 ± 0.2	4.9 ± 0.4	5.3 ± 0.5	6.5 ± 0.5
Growth rate (mm/day)	0.9 ± 0.07	0.6 ± 0.03	0.9 ± 0.03	0.7 ± 0.02
Day of onset of atresia	3.5 ± 0.1[a]	4.0 ± 0.1[b]	14.7 ± 0.2[c]	16.8 ± 0.2[d]
Persistence (days)	4.8 ± 0.6	5.1 ± 0.5	4.1 ± 1.1[a]	7.7 ± 0.4[b]

*Day 0 is the day of ovulation; [a, b, c, d] Values followed by different superscripts within rows differ at $P<0.05$ and $P<0.01$, respectively.

Discussion

Lameness, a chronic form of stress, affects reproduction in dairy cows (HERNANDEZ et al., 2005). In the present study, follicular dynamics were determined throughout the estrous cycle in the lame cows (L) and compared with the non-lame cows (N). To our knowledge this is the first comprehensive description of follicular dynamics in lame cycling cows.

Lameness did not appear to affect the follicular characteristics at the emergence of anovulatory and ovulatory waves in the N and the L cows (Table 1). However, certain variations were recorded with the progression in follicular waves. One of the most striking differences was the higher ($P<0.01$) number of medium-sized follicles during the second half of the anovulatory wave in the L cows (Fig. 1; middle panel). This phenomenon may be associated with a reduced dominance of DFs, thereby permitting the growth of SFs arising in the same cohort. A decrease in the number of medium sized follicles is considered to be a sensitive indicator of follicular dominance (BEG et al., 2001). Moreover, the prolonged sustenance and growth of the medium sized follicles enabled some of these to become large-sized follicles and increase in number during the anovulatory wave in the L cows (Fig. 1; top panel, $P<0.10$). The follicular status was more disturbed during the ovulatory wave as was evident from the even higher number of medium- and large-sized follicles in the L cows (Fig. 1) suggesting a greater probable reduction in follicular dominance during the ovulatory wave. Hence, lameness appeared to suppress the dominance of the anovulatory and ovulatory DFs during 2-wave cycles. Under-nutrition (DISKIN et al., 2003), heat stress (ROTH et al., 2000) and lactation (WOLFENSON et al., 2004) are the other chronic stressors known to affect the pattern of follicular development during a wave. Although not recorded in the present study, lame cows spend more time lying down to avoid pain from walking or standing, which may predispose the cows to chronic dietary restrictions (GALINDO and BROOM, 2002). The plasma concentrations of NEFA, a reasonable indication of nutrient status in ruminants (ADEWUYI et al., 2005), were within physiological limits (KANEKO et al., 1997) and did not differ between the N and L cows, which rules out under-nutrition affecting follicular dynamics in the L cows. The identical environmental and postpartum lactation also exclude the effects of heat stress and lactation on follicular dynamics in the L cows.

Lameness did not have any apparent effect on the daily average size of the anovulatory DF and the OF. However, the tendency of treatment-by-day interaction ($P = 0.10$) for the average size of OF may be attributed to the slower growth rate of the OFs in the L cows (Fig. 2).

Selection of the anovulatory DF and the OF on day 2.8 ± 0.1 in the N cows corroborates with a previous study (GINTHER et al., 2003), whereas it was delayed significantly to day 3.6 ± 0.2 and day 5.6 ± 0.2 respectively, in the L cows. Selection is associated with an increase in estradiol and inhibin production that reduces circulating FSH and the resultant atresia of follicles in the same cohort (BEG et al., 2001). Conversely, low estradiol and inhibin during a delayed selection of follicles provides extended FSH support for growth of SFs in the same cohort (GINTHER et al., 1996); a similar phenomenon has been documented in cows under chronic heat stress (ROTH et al., 2000). Whether delayed follicle selection, delayed onset of atresia of the largest SFs ($P<0.05$ at least; Table 1) and

increased number of small, medium and large - sized follicles ($P<0.05$ at least; Fig. 1) in the L cows are associated with similar endocrine aberrations, warrants investigation.

Persistence of the OF in the N cows (9.5 ± 0.2 days) was similar to a previous report (GINTHER et al., 1989a), but was comparatively longer ($P<0.05$) in the L cows (10.9 ± 0.5 days). Increased persistence of the OFs may affect conception rate. Under normal circumstances, OFs arising from 2-wave cycles persist longer and yield 1.5 day older ovum compared to 3-wave cycles. Consequently, significantly higher conception rates have been reported in the cows exhibiting 3-wave follicular growth during an estrous cycle (SAKAGUCHI et al., 2004; TOWNSON et al., 2002). Hence, the increased persistence of the OF in the L cows (especially six out of thirteen L cows having OF persisting for 11 to 13 days) may be the reason for reduced conception rates in lame cows quoted elsewhere (HERNANDEZ et al., 2005). Increased persistence of the follicles reduces the fertilization and development competence of oocytes in bovines (ROTH et al., 2008).

Conclusions

In conclusion, the findings of the present study supplement the literature that certain aspects of follicular dynamics are altered in lame cycling cows having 2-wave follicular growth, which need to be corroborated with fertility and endocrine changes governing follicular dynamics.

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SAŽETAK

Hromost utječe na rasplođivanje mliječnih krava. Dosada nije ustanovljeno utječe li i na folikularnu dinamiku u tijeku spolnoga ciklusa. Ultrazvučna pretraga jajnika provedena je jednom dnevno u jednom ili dvama međuovulacijskim razmacima u normalnih i hromih krava. Pretežito su ustanovljena dva vala folikularnog rasta te je to uzeto za usporedbu kretanja folikularnog rasta između šest i 13 međuovulacijskih razmaka u šest normalnih i 10 hromih krava. Značajke pojave folikularnih valova bile su slične u normalnih i hromih krava. U odnosu na normalne, hrome krave pokazivale su povećani broj malih ($P<0,05$), srednjih ($P<0,01$) i velikih ($P<0,05$) folikula u tijeku valova ovulacijskih folikula, zatim zakašnjelu selekciju dominantnog folikula u izvanovulacijskom (dan $3,6 \pm 0,2$ u odnosu na dan $2,8 \pm 0,1$; $P<0,05$) kao i ovulacijskom valu ($5,6 \pm 0,2$ dana u odnosu $2,8 \pm 0,1$ dana; $P<0,01$) i veću postojanost ovulacijskog folikula (10,9 u odnosu na 9,4 dana; $P<0,05$). Može se zaključiti da se sastav i značajke folikula mijenjaju u hromih krava.

Ključne riječi: folikularni valovi, hromost, križane krave