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Effect of lameness on follicular dynamics in postpartum anestrous crossbred cows

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ABSTRACT

Lameness affects the reproductive performance of cows. It is not clear whether follicular dynamics is involved. Ovarian ultrasonography was performed once daily in eight non - lame (N) and 11 lame (L) cows for a period of 30 days each. Recurrent follicular waves could be detected every 8.3 ± 0.4 and 7.6 ± 0.5 days, respectively, in the N and L cows. There were no appreciable differences in the day of selection (Day 2.3 ± 0.2 versus Day 2.8 ± 0.2), maximum diameters (8.9 ± 0.6 mm versus 9.2 ± 0.5 mm) and certain characteristics pertaining to growth, static and regression of the dominant follicles (DFs) in the N versus the L cows. The L cows, however, had a tendency to have a higher number (P<0.10) as well as treatment - by - day interaction (P<0.05) for the identified (detectable for more than one consecutive day) and unidentified (detectable for one day only) small follicles (>3≤5 mm). In conclusion, lameness did not affect the DFs that developed but failed to ovulate, but more the number of small follicles, both identified and unidentified, hints at some perturbance in the follicular characteristics of lame cows that needs to be addressed in the light of the mechanisms governing follicular dynamics.

Key words: follicular dynamics, postpartum anestrus, lameness, crossbred cows

Introduction

Crossbreeding initiated in several tropical countries to augment milk production has met with limited success (DESHMUKH and KAIKINI, 1999). The increased vulnerability of crossbred cows to certain diseases (LOSSOS, 1986) including lameness has been one of the main reasons. The incidence of lameness in dairy cows is increasing in the tropics (SOOD, 2005) as well as in Europe (ESPEJO et al., 2006). The reproductive potential of lame cows has been indicated to be below par (MELENDEZ et al., 2003; SOOD, 2005), and an extended calving to conception interval (HERNANDEZ et al., 2005) is one of the

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major concerns. Therefore understanding the pattern of follicular development during the anestrous period is imperative in lame cows. The purpose of the present investigation was to characterize the changes in the ovarian follicles on a comparative basis between normal anestrous (N) and lame anestrous (L) postpartum cows.

Materials and methods

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

Animals and management. The study was conducted at the Dairy Farm of Punjab Agricultural University, Ludhiana, India. The animals were housed loose and fed *ad lib* with seasonal green fodder, wheat straw, silage and an adequate quantity of concentrate ration (ANONYM., 2001).

Scoring of lameness and selection of cows. A total of 19 normally calved crossbred dairy cows (Holstein Friesian × Sahiwal; exotic inheritance of 50.0 to 62.5%) were investigated after breeding period.

During the study, 11 cows with apparent lameness were scored for severity of lameness using a five-point lameness scale (WELLS et al., 1993). The cows were lame either due to interdigital dermatitis resulting from some physical etiology (n = 6) or hock joint arthritis manifested by a painful swelling either with (n = 4) or without (n = 1) a wound in the affected region.

Before initiating the study, all the lame cows received i.m and b.i.d a five day course of Streptomycin sulfate at a rate of 6-8 mg kg^{-1} and Procaine penicillin G at a rate of 6000 - 7000 I.U kg^{-1} contained in a single preparation [Munomycin®, India] alongwith Meloxicam at a rate of 0.2 mg kg^{-1} [Melonex™, India]. Simultaneous to the systemic treatment, the lesions causing lameness were treated locally by cleaning with warm 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.]. All the lame cows at the time of initiation of the study had a moderate degree of lameness (score of 2; characterized by moderate and consistent gait asymmetry or symmetric gait abnormality, but able to walk without continuous stimulation). Investigations on follicular dynamics in the lame cows were initiated 8 to 10 days after termination of systemic treatment. During the period of study, the lame cows were treated locally as mentioned *vide supra* for a period of 10 to 12 days. Another six non-lame (N) herd mates (score of 0) were included for control observations. Each of the selected normal and lame cows was scored for presence and severity of lameness every week during the course of the study. Lameness scoring was always performed by the first author by observing the cows walking on a 20 m firm walkway following evening milking.

The reproductive status of the cows was ascertained from history and gynecological examination involving ultrasonography and transrectal palpation of the genitalia. All the cows remained anestrus throughout the study. At the start of experiment, the lactation number, postpartum stage, body mass and body condition score (BCS, along a scale of 1 to 5 using 0.25 unit increments) (EDMONSON et al., 1989) were recorded. Milk yield in the selected cows during the course of study was also recorded.

Follicular dynamics. All the cows were subjected once daily to ovarian follicular 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 were made for a period of 30 days following confirmation of the absence of a CL.

During each examination the number, size and location of antral follicles of at least 3 mm in diameter were recorded. The components [dominant follicle(s) DF(s); subordinate follicle(s) SF (s)] and characteristics [growing, static and regressing phases, persistence of the DFs and SFs] were determined as per GINTHER et al. (1989). Selection of a DF was as defined by GINTHER et al. (1996); the average values in the N and L cows are presented. Follicles detected for at least two consecutive days were designated as identified follicles, whereas the ones detected only for one day were designated as unidentified follicles. The percentage of unidentified small, medium and large follicles were determined for the first six days from onset of follicular wave.

Due to the lower maximum diameters of the follicles in the crossbred cows of the present study compared to cows raised in Europe (GINTHER et al., 1989) the follicles had to be categorized into three sizes, $>3\leq 5$, $>5\leq 7$ and >7 mm, that were considered as small, medium and large, respectively. The DF was of at least 7 mm size and exceeded the diameter of all the follicles arising in the same cohort.

Blood samples. A total of four heparinized blood samples were collected through direct jugular venupuncture at weekly intervals from each cow and were immediately placed on ice; the samples were centrifuged at 2500 g for 15 min within 1h and the plasma was stored at -20 ^{o}C. The plasma was utilized to estimate the concentration of peripheral progesterone (KAMBOJ and PRAKASH, 1993) and non-esterified fatty acids (NEFA) (FOLCH et al., 1957). The blood samples were always collected 3 to 5 h after morning feeding between 1200 and 1300 h.

Statistics. The growth and regression rate (mm/d) of each DF was calculated by linear regression (slope = growth and regression rate) and the mean growth or regression rate of DFs in each group was determined.

The growth, static and regressing phases, as well as the selection and persistence of the DFs, the maximum diameter and persistence of the SFs between the N and the L cows were compared by Student's *t*-test.

The sizes of the DFs and the total number of small, medium and large follicles were analysed by the Statistical Analysis System general linear models univariate analysis of variance of repeated measures with degrees of freedom, modified by the Green house-Geyser epsilon (SAS/STAT™ 9.1.3.). The statistical model included the effect of treatment (N versus L cows), the cow (within treatment), the day - effect and treatment - by - day interaction.

The percentage of unidentified follicles of different sizes was determined for the first six days of all the waves in the N and the L cows, except for one wave that lasted for 3 days in an L cow. Data for small unidentified follicles were analysed for treatment - by - day interaction using repeated measurement analysis. Larger diameters were not analysed because of the smaller proportion of unidentified follicles.

The plasma NEFA concentrations were compared between the N and the L cows by Student's *t*-test, whereas the plasma progesterone concentration was used for confirmation of anestrus and were not compared statistically.

Results

The moderate degree of lameness at the initiation of investigation of follicular dynamics had improved to a mild form (score of 1; characterized by mild gait asymmetry and/or restriction in free movement) by the end of the study in all the lame cows.

The average (range) lactation number 3.4 ± 0.2 (4 to 5), postpartum stage 79.8 ± 3.1 days (72.0 to 89.0 days), BCS 3.1 ± 0.3 (3.0 to 3.5), body mass 410.2 ± 7.2 kg (392 to 444 kg) and daily milk yield 8.2 ± 1.9 l/d (8.3 to 13 l/d) did not differ (P>0.05) between the N and L cows.

The plasma progesterone concentrations fluctuated between 0.4 and 0.8 ng/mL which corroborated to the presence of anestrus in the N and the L cows.

Follicular dynamics in the N and the L cows. During the 30 day study period a total of 23 and 33 complete waves could be tracked in the N and the L cows, respectively.

Follicular waves occurred at every 8.3 ± 0.4 and 7.6 ± 0.5 days, respectively, in the N and L cows. Each follicular wave in the N and the L cows was characterized by the presence of a group of follicles that emerged together, but only one of these follicles continued to increase in size to become a DF. The DF attained a maximum diameter of 8.8 ± 0.6 mm and 9.6 ± 0.5 mm in the N and the L cows, respectively. The characteristics of the DF pertaining to growth, static and regressing phases and persistence did not differ between the N and the L cows (Table 1).

Table1. Certain characteristics of the anovulatory follicular waves in normal (23 waves in eight cows) and moderately lame (33 waves in 11 cows) postpartum anestrous cows

Characteristic*	Normal		Lame	
	Mean ± s.e.m	Range	Mean ± s.e.m	Range
Inter-wave interval (days)	8.3 ± 0.4	6.0 to 10.0	7.6 ± 0.5	3.0 to 12.0
Follicles detected at initiation of wave (N^o)	2.9 ± 0.1	2.0 to 5.0	2.8 ± 0.2	1.0 to 6.0
Dominant follicle				
Growth phase				
Diameter at first detection (mm)	3.4 ± 0.1	3.1 to 4.2	3.2 ± 0.2	3.2 to 3.9
Linear growth rate (mm/day)	1.1 ± 0.2	0.6 to 1.9	1.0 ± 0.1	0.7 to 2.0
Maximum diameter (mm)	8.9 ± 0.6	7.1 to 16.8	9.2 ± 0.5	7.0 to 13.6
Days taken to attain maximum diameter	5.3 ± 0.1	4.0 to 6.0	6.2 ± 0.4	3.0 to 6.0
Static phase (days)	3.6 ± 0.2	3.0 to 5.0	3.9 ± 0.3	3.0 to 6.0
Regressing phase				
Linear regression rate (mm/day)	-1.0 ± 0.3	0.7 to 2.1	-1.0 ± 0.05	0.6 to 2.3
Persistence (days)	13.3 ± 1.3	10.0 to 15.0	14.4 ± 1.2	9.0 to 14.0
Largest subordinate follicle				
Maximum diameter (mm)	4.5 ± 0.2	4.1 to 6.1	5. 2 ± 0.3	4.3 to 5.9
Persistence (days)	3.9 ± 0.4	3 to 6	4.6 ± 0.2	3.0 to 6.0

* None of the characteristics differed (P>0.05) between the normal and lame cows

The average size of the DFs on different days of the wave was similar between the N and the L cows and was not influenced (P>0.05) by the effect of treatment, the cow, day - effect and treatment - by - day interaction (Fig. 1). The selection of the DFs occurred on Day 2.3 ± 0.2 and Day 2.8 ± 0.2, respectively, in the N and the L cows. The diameter of the DF and the associated largest SF on the day of selection was 5.5 ± 0.1 mm and 5.0 ± 0.2 mm in the N cows and 4.9 ± 0.3 mm and 4.5 ± 0.2 mm in the L cows.

The L cows tended to have a higher number of small follicles (P<0.10). A treatment - by - day interaction (P<0.05) was detected for the small follicles (lower panel Fig. 2) but not for the medium and large - sized follicles (middle and upper panels, Fig. 2).

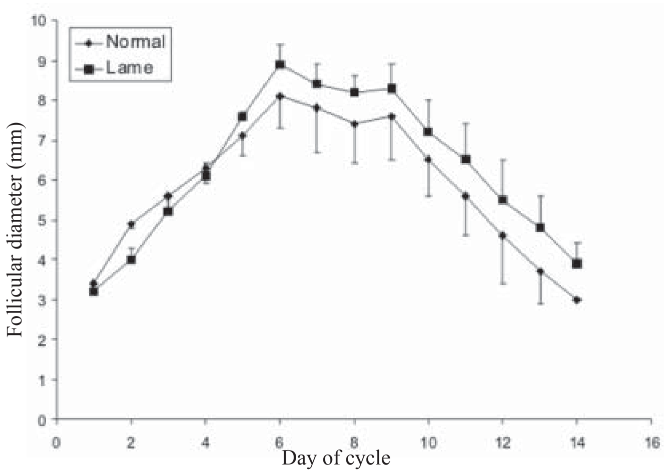

Fig. 1. Growth patterns of the dominant follicles during anovulatory waves in normal anestrous (n = 8; follicular waves = 23, solid triangles) and lame anestrous (n = 11; follicular waves = 33, solid squares) crossbred cows

The percentage of unidentified follicles of small, medium and large sizes was 96.0, 4.0 and 0 in the N cows and 94.0, 5.5 and 0.5 in the L cows, respectively. A treatment - by - day interaction was detected (P<0.05) for the number of unidentified small follicles. The number of unidentified small follicles was similar during each day of the six day period of the wave (P>0.05), however, it tended to be higher (P<0.10) when considered for the entire wave (Fig. 3).

The average concentration of NEFA did not differ (P>0.05) between the N (93.2 ± 2.6 mg/L) and L (86.2 ± 1.9 mg/L) cows.

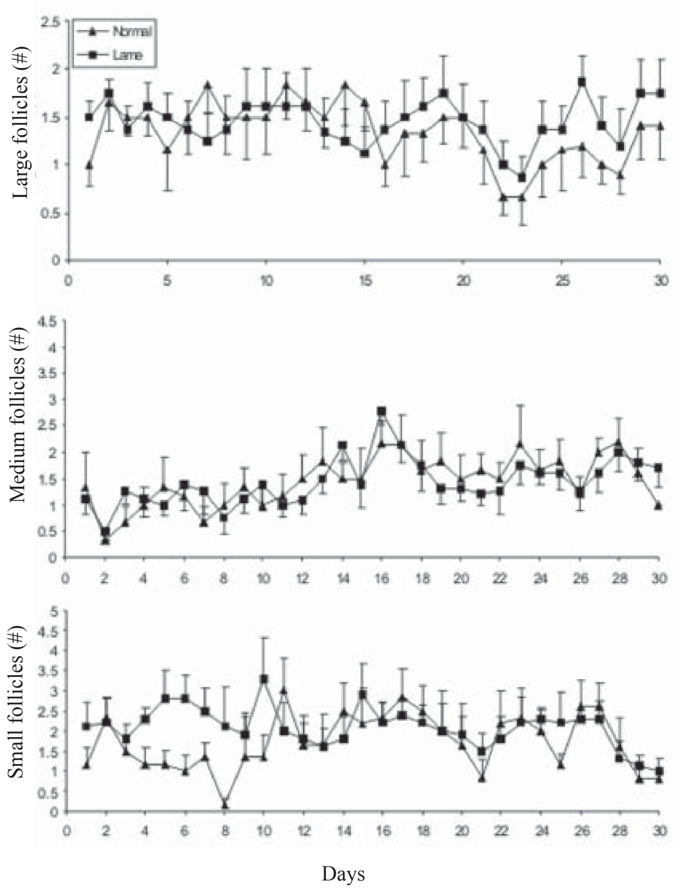

Fig. 2. Number of small (>3≤ 5 mm), medium (>5≤7 mm) and large (>7mm) follicles during the 30 day study period in normal anestrous (n = 8; follicular waves = 23, solid triangles) and lame anestrous (n = 11; follicular waves = 33, solid squares) crossbred cows

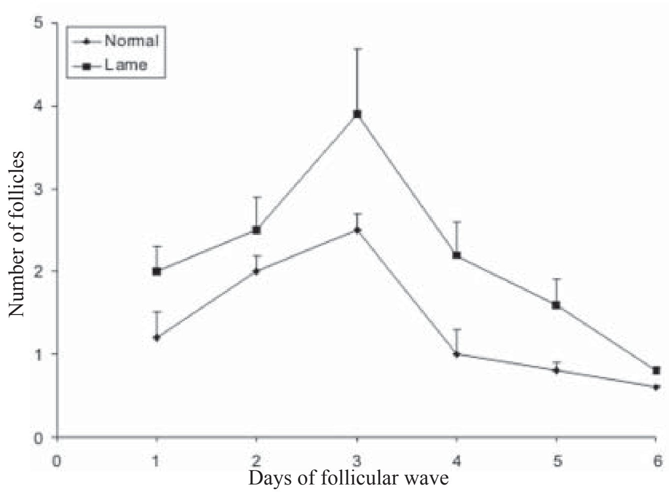

Fig. 3. Mean ± s.e.m of number of unidentified small follicles (>3≤ 5 mm) recorded during the first six days of follicular wave in the normal anestrous (n = 8; follicular waves = 23, solid triangles) and lame anestrous (n = 11; follicular waves = 32, solid squares) crossbred cows

Discussion

To our knowledge this is the first comprehensive report on follicular dynamics in postpartum lame cows.

In today's livestock production systems, characterized by narrow profit margins and ever-increasing pressure to augment productivity, the reproductive performance of individual animals is important. Lameness has been recognized as a serious deterrent to dairy economics through reduced reproductive and production potential and increased culling rate of the affected cows (ESPEJO et al., 2006). A delay in postpartum ovarian activity has been recorded in lame cows (MELENDEZ et al., 2003); however, the status of follicular dynamics in the postpartum anestrous lame cows is not known.

All the cows in the N and the L groups exhibited a wave pattern of follicular growth and regression at every 8.3 ± 0.4 and 7.6 ± 0.5 days, respectively. Apparently longer interwave intervals of 9.7 ± 0.5 days (MURPHY et al., 1990) and 9.9 ± 1.1 days (HENAO et al., 2000) have been reported in postpartum anestrous cows. The duration of the interwave interval in the present study was directly related to the maximum size of the DF (r = 0.4, P = 0.05), which is substantiated by the smaller maximum diameters of the DFs in the N (8.8 ± 0.6 mm) and the L (9.6 ± 0.5 mm) cows and a shorter interwave interval compared to the larger follicular diameters in cows raised in Europe (10.8 to 15.4 mm, MURPHY et al., 1990; OLIVEIRA et al., 2002) and a longer interwave interval as mentioned *vide supra*.

A similarity in the growth, static and regression phases, persistence (Table 1) as well as the average day to day changes in the maximum diameters (Fig. 1) of the DFs in the N and the L cows was not suggestive of any significant influence of lameness on the characteristics of the DFs. Contrary to our findings, cows with lameness and other production diseases have been reported to possess smaller follicles than their healthy counterparts at 6 weeks postpartum (W. J. CLARKE, H. DOBSON and R. F. SMITH, unpublished, cited in DOBSON et al., 2003). This variation could be due to a difference in the postpartum stage or other factors related to lameness such as severity, lesions, chronicity of the disease or other factors. It is, however, pertinent to mention that the maximum diameters of the DFs in the N and the L cows resembled the anovulatory DFs (9.5 ± 0.8 mm) but were, however, smaller ($P<0.01$) than the ovulatory DFs (12.6 ± 0.2 mm) in normal (non-lame) cycling cows investigated from within the same herd in a separate study by us (SOOD et al., 2009).This suggests that although the DFs underwent a normal process of selection as found in cycling cows (GINTHER et al., 2003), the impetus required for final follicular maturation (GINTHER et al., 2003) was missing in both the N and the L cows.

The number of follicles at the initiation of the wave and characteristics of DFs in the N and the L cows were similar (Table 1), nevertheless the average number of small - sized follicles per day tended to be higher ($P = 0.10$) in the L (2.0 ± 0.09) than the N (1.7 ± 0.1) cows; the numbers of medium (1.6 ± 0.07 versus 1.4 ± 0.08) and large (1.4 ± 0.04 versus 1.3 ± 0.05) follicles were, however, similar in the L versus the N cows, respectively. The variation in the number of small follicles resulted in a significant treatment - by - day interaction in the L cows (Fig. 2). A detailed analysis revealed the later interaction to be a repercussion of the relatively longer persistence of follicles in the L cows. The average persistence of all follicles, other than the DFs, was longer ($P<0.05$) in the L (4.4 ± 0.1 days) than the N (4.0 ± 0.1 days) cows. Chi - square analysis (unplanned) revealed a higher number ($P<0.05$) of follicles persisting for five days in the L cows (25 out of 62 - 40.3%) than the N cows (12 out of 46 - 26.1%). In the latter cows majority follicles persisted for either three (15 out of 46 - 32.6%) or four (16 out of 46 - 34.8%) days. Heat stress has been reported to reduce the dominance of DFs thereby permitting the growth and increase in the number of subordinate follicles in the cycling cows (ROTH et al., 2000). As the selection of the DFs did not differ between the N and the L cows (Day 2.3 ± 0.2 and Day 2.8 ± 0.2, respectively), the phenomenon of altered dominance of the DFs could not be established as the probable reason for the extended persistence of the follicles in the L cows. Lameness is a stressful situation (GALINDO and BROOM, 2002) that could alter the functioning of the hypothalamo - pituitary axis as revealed by the indifferent response to certain exogenous hormones in the lame compared to normal cows (SOOD, 2005). Whether the endocrine mechanisms dictating follicular dynamics are also altered in lame cows need to be investigated.

The higher number of unidentified small follicles (P<0.01) during the six days of the wave (Fig. 3) contributed to a significant treatment - by - day interaction in the L cows. A trend of increase and decrease in the number of these follicles during the entire six day period was identical in both the groups. The relatively higher number of unidentified small follicles on the second, third and fourth days than on the other days of the wave suggest that these may emerge in response to the endocrine support responsible for the initiation of a follicular wave, but the failure of the majority of small follicles to become medium or large follicles may be due to the process of selection of DF, resulting in atresia of the subordinate follicles. Not many studies have reported about unidentified follicles, the available literature suggests the presence of 2.1 ± 0.5 to 4.9 ± 0.7 unidentified follicles during the anovulatory and ovulatory waves in cycling cow heifers (GINTHER et al., 1989). As recorded in the present study, lame cows exhibited a higher number of unidentified follicles, which is also supported by results from another experiment on lame cycling cows who also had a higher number (P<0.01 (data not presented) of unidentified follicles (SOOD, 2005). Several intrinsic and extrinsic endocrine factors regulating the follicular growth (NILSSON et al., 2002) need to be addressed in lame cows.

Although not recorded in the present study, lame cows spend more time lying down so as to avoid pain from walking or standing for an extended period, which may predispose the cows to chronic dietary restrictions (GALINDO and BROOM, 2002). Under-nutrition could affect the growth rate, diameter and persistence of the DFs (DISKIN et al., 2003). Plasma concentrations of NEFA give a reasonable indication of nutrient status in ruminants (ADEWUYI et al., 2005). The concentrations of NEFA were observed to be within physiological limits (KANEKO et al., 1997) and did not differ between the N and L cows, which rules out the lack of nutrition as the overriding cause of altered follicular dynamics in the lame cows.

Conclusion

In conclusion, the presence of lameness did not affect the characteristics of the follicles at the origin of the wave, selection and the growth as well as regressing phases of the dominant follicles. The dominant follicles developed but failed to ovulate in both the normal and the lame cows. The lame cows, however, tended to have a higher number of identified as well as unidentified small follicles which needs to be substantiated with the mechanisms dictating follicular dynamics.

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SAŽETAK

Hromost utječe na reprodukcijsku sposobnost krava, ali nije sa sigurnošću ustanovljeno utječe li i na folikularnu dinamiku. Jajnici osam normalnih i 11 hromih krava ultrazvučno su bili pregledavani jednom dnevno u razdoblju od 30 dana. Povratni folikularni valovi mogli su se ustanoviti svakih 8,3 ± 0,4 u normalnih i 7,6 ± 0,5 dana u hromih krava. Nisu bile ustanovljene znatne razlike u selekciji (dan 2,3 ± 0,2 u normalnih, 2,8 ± 0,2 u hromih), najvećem promjeru (8,9 ± 0,6 mm u normalnih, 9,2 ± 0,5 mm u hromih) određenim značajkama rasta, postojanosti i regresije dominantnih folikula u normalnih u odnosu na hrome krave. Međutim hrome krave pokazivale su tendenciju većeg broja (P<0,10) na dan interakcije (P<0,05) za identificirane (dokazivi tijekom više od dva uzastopna dana) i neindentificirane (dokazivi samo tijekom jednog dana) male folikule (>3≤5 mm). Zaključuje se da hromost nije utjecala na dominantne folikule koji su se razvili, a nisu ovulirali, ali veći broj malih folikula, identificiranih i neidentificiranih, upućuje na neke smetnje folikularnih značajki u hromih krava koje se moraju uzeti u obzir u svjetlu mehanizma što upravlja folikularnom dinamikom.

Ključne riječi: folikularna dinamika, poslijeporođajni anestrus, hromost, križane krave