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—Original Article—

A pre-synchronization program at early postpartum might increase the chances of *Bos indicus* cows cycling prior to 50 days regardless of the length of calf separation

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Abstract. The aim of this study was to establish if pre-synchronization would enhance the number of animals cycling prior to conventional breeding at 45 days irrespective of the length of calf separation. Multiparous *Bos indicus* cows were allotted in four groups (n = 10). Control group (C) dams remained with their calves; groups G24, G48 and G72, which were partially weaned for 24, 48 and 72 h, respectively, were estrus synchronized using a controlled internal drug. These procedures were performed at 25 days and again at 45 days postpartum. The number of follicles, presence of a corpus luteum and back fat thickness (BFT) were determined by ultrasound. The proportion of cows with estrus and ovulation at day 25 postpartum was statistically different between the control and treated groups, with the values being 20, 60, 50 and 70 for the control, G24, G48 and G72 groups respectively ($P < 0.05$). At days 45 postpartum, the proportion of cows with estrus and ovulation was different in group G48 compared with the other groups ($P < 0.05$). The average BFT and body condition score for the four experimental groups in the two periods were similar ($P > 0.05$). Animals with a higher proportion of follicles from 17 to 21 mm, BFT values above 3.5 mm and a regular body condition were significantly different regardless of whether the dams remained with their calves or were separated, regardless of the length of this event. It can be concluded that (1) a pre-synchronization program at day 25 could trigger the onset of ovarian activity and facilitate a breeding program at day 50 and (2) temporary weaning enhances the effect of a pre-synchronization program.

Key words: Anestrus, Breeding program Ovarian activity, Temporal weaning, Zebu

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The onset of ovarian activity in Zebu cattle appears to be a major hurdle in achieving calving intervals within a range of 12–14 months [1]. The establishment of this event is particularly important in systems in which seasonal breeding is the norm. On the other hand, the effect of suckling has been suggested to be the most important obstacle to re-initiation of a fertile estrous cycle; thus a considerable amount of research has been vested in this topic [2, 3]. This is in spite of the fact that follicular turnover appears to start soon after parturition [4]. In effect, several reports indicate that there are instances in which Zebu animals displayed behavioral estrus and ovulation before 60 days postpartum [5–7].

The methods of calf separation and the time postpartum at which this manipulation occurs vary, and consequently, it is difficult to form an understanding based on the data from these reports. In general, there is agreement that poor body condition after calving, scarce fodder availability and the frequency of suckling are driving factors impairing prompt restoration of ovarian activity. Among the methods for shortening the onset of ovarian activity is separation of the calf from the dam for various periods of time [8, 9]. There is some controversy concerning whether one system is better than another as there are few controlled studies on this subject [10].

The hypothesis tested in this study was that the first synchronization period would promote the restoration of ovarian activity and the onset of estrus but result in subpar ovulations. Thus, facilitating the regularity of estrous cycles and hence promoting a second synchronization would result in estrus and a consistent number of ovulations.

The objective of the present study was to test if a pre-synchronization program at day 25 could trigger the onset of ovarian activity and facilitate a breeding program at day 50 regardless of the system of calf separation.

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Materials and Methods

The study was undertaken at the Centre for Teaching, Research and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine of the National Autonomous University of Mexico, which is located in the state of Veracruz, Mexico, at 20º 4´N and 97º 3´W. The climate is hot and humid, without a defined dry season. The average yearly rainfall is 1840 mm, and the average temperature ranges between 14 and 35 C.

Animals and management

Forty multiparous *Bos indicus* cows were used. All calved between May and June of 2013; the interval from the first to last animal calving was 61 days. All cows were kept under grazing land conditions in a *Cynodon nlemfuensis* pasture from June to September. The average rainfall for these months was 674 mm, and average temperature was 26 C.

The 40 cows were allotted to four groups: control (C) dams remained with their calves; temporary weaning was performed in groups G24, G48 and G72 for 24, 48 and 72 h respectively. Calves were separated from their dams in two periods, which started at days 25 and 45, in groups G24, G48 and G72. At the end of each temporary weaning period, the calves were returned to their dams. The calves and cows could see each other, but a fence impeded physical contact and suckling as described previously [11].

All cows were estrus synchronized twice, at day 16 and day 36 after calving, using a controlled internal drug release device (CIDR, Eazi-Breed, 1.9 g natural progesterone, Pfizer®, Mexico), which remained *in situ* for 9 days. The CIDR device was withdrawn on days 25 and 45 respectively. In groups G24, G48 and G72, temporary weaning started at the time of CIDR withdrawal.

To determine the number of follicles and presence of a corpus luteum (CL), ultrasonography was performed on all cows using a real-time B-mode linear array ultrasound scanner with a 7.5 MHz rectal probe (Aloka SSD-500, Tokyo, Japan). Number of follicles was determined every day for four days after CIDR withdrawal in the two periods. Follicles were classified into three categories: 7 to 11 mm, 12 to 16 mm and 17 to 21 mm. The presence of a CL was determined by ultrasound at days 9 and 11 after CIDR withdrawal.

Blood samples were obtained from the coccygeal vessels, at days 9 and 11 after CIDR withdrawal in the two periods using Vacutainer® tubes without an anticoagulant. Blood samples were centrifuged at 3000 RPM for 30 min. Serum was stored in 1.6 ml Eppendorf vials at –20 C. Progesterone concentrations were determined using a commercial solid phase RIA kit (Progesterone, Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA; batch TGC2 0417) validated for bovine. The intra-assay coefficient of variation was 6.9%. Values ≥ 1 ng/ml were indicative of the presence of a CL and were also an ovarian activity indicator [12].

Cows were observed for 96 h after CIDR withdrawal. Observations started at 0700 h and finished at 1900 h. With the aim of identifing cows that were in estrus after 1900 h, ESTROTECT patches (Rockway, Spring Valley, WI, USA) were placed on all cows. Changes in the color of the patches were registered every morning at 0700 h.

Back fat was measured for all cows at days 16, 38 and 58 postpartum using an Aloka SSD-500 ultrasound with a 3.5 convex probe on the back side of each cow and between the tuberosity of the ileum and the ischium [13].

Body condition was assessed using a scale from 1 to 9 [14] at the same time as measurement of back fat.

Statistical analysis

Statistical analysis was performed for the variables back fat thickness and body condition using ANOVA for repeated measures over time (at 16, 38 and 58 days postpartum) in a 2 × 2 factorial arrangement with factors such as estrus plus ovulation as respondent variables. Furthermore, correlation analyses were performed using variables such as follicle size, body condition score and back fat and taking into consideration the effect of time (25 to 45 days) using the JMP 5.0.1 and SPSS 16 statistical software. Values were considered statistically significant at $P < 0.05$. A comparison between treatments was undertaken using a test of proportions [15] for the following variables: a) estrus and ovulation, b) estrus but no ovulation, c) no estrus and ovulation and d) no estrus and no ovulation.

Results

The proportion of cows with estrus and ovulation at day 25 postpartum was smaller in the control group when compared with the treated groups, with the values being 20, 60, 50 and 70% for the C, G24, G48 and G72 groups respectively ($P < 0.05$). The average time of onset of estrus for cows ovulating at day 25 was 58.2 ± 3.1 h after CIDR withdrawal for all groups, whilst the value for day 45 was 68.9 ± 2 h. No significant differences were found for time of onset of estrus between the two periods ($P > 0.05$). The percentage of cows displaying estrus behavior and ovulation at 45 days postpartum was larger in group G48 when compared with the other groups, with the values being 50, 40, 80 and 50% for C, G24, G48 and G72, respectively (Table 1).

Overt signs of estrus were not observed in 20% of the cows in each of the four groups at day 25, but these cows ovulated nonetheless as revealed by progesterone values ≥ 1 ng/ml. In contrast, at day 45 postpartum a higher number of cows separated from their calves for 24 h displayed behavioral estrus but ovulated (40%) than those cows separated from their calves for 72 h (20%). In the control and G48 groups, h, only one cow per group did not display estrus accompanied by ovulation. The number of cows showing estrus at day 25 but not ovulation was similar in all groups (one cow out of ten for the G24 and G48 groups and none for the C and G72 groups). At day 45, this condition was detected in only one out of ten cows in the C, G24 and G72 groups and in none of the cows in the G48 group (Table 1).

The proportion of cows neither ovulating nor displaying estrus at day 25 was larger in the control group than in the treated groups, with the values being 60, 10, 20 and 10% for the C, G24, G48 and G72 groups respectively ($P < 0.05$). The values for day 45 were similar for all groups, that is, 30, 10, 10 and 20% respectively ($P > 0.05$; Table 1).

As can be observed in Fig. 1, only one out of 10 cows in the C group at day 25 postpartum had a follicle > 17 mm during the intense ultrasound examinations. This was significantly smaller ($P < 0.05$) in comparison with the G24, G48 and G72 groups, in

Table 1. Proportion of cows displaying estrus and ovulation at 25 and 45 days pospartum in the control group (C) and in the groups after being subjected to temporary weaning at 24 h (G24), 48 h (G48) and 72 h (G72)

Group	25 days postpartum					45 days postpartum				
	Onset of estrus (h)	Estrus and ovulation	Estrus and no ovulation	No estrus and ovulation	No estrus and no ovulation	Onset of estrus (h)	Estrus and ovulation	Estrus and no ovulation	No estrus and ovulation	No estrus and no ovulation
C	74.0 ± 0.0	20 [b]	0	20	60 [b]	73.6 ± 1.1	50 [b]	10	10 [b]	30
G24	58.6 ± 2.9	60 [a]	10	20	10 [a]	72.0 ± 0.8	40 [b]	10	40 [a]	10
G48	49.9 ± 1.6	50 [a]	10	20	20 [a]	63.5 ± 3.4	80 [a]	0	10 [b]	10
G72	51.0 ± 1.6	70 [a]	0	20	10 [a]	66.8 ± 3.1	50 [b]	10	20 [b]	20

Values are expressed as percentages. [a, b] Different letters indicate differences within the same column at $P < 0.05$.

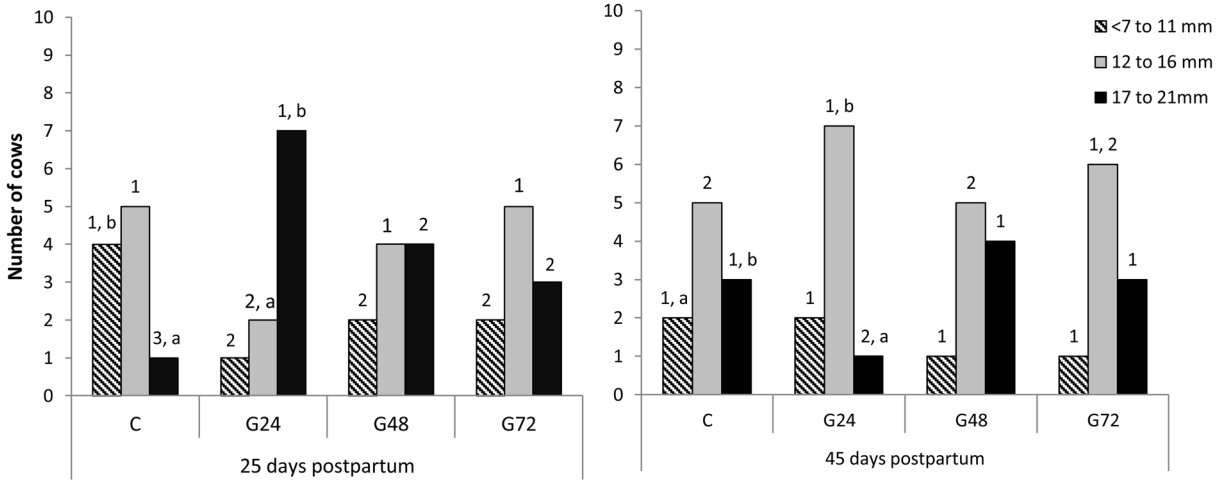

Fig. 1. Follicular diameters of cows at days 25 and 45 postpartum. The black bars represent follicles from females with theoretical ovulatory capacity. [1,2,3] Different numbers represent differences in follicular categories among groups at $P < 0.05$. [a,b] Different letters indicate differences between follicular categories between 25 and 45 days postpartum at $P < 0.05$.

which 7, 4 and 3 cows presented follicles above 17 mm respectively, suggesting delayed development in follicles likely to be ovulated. In the latter groups, a higher number of females in the G24 group than in the G48 and G72 groups ($P < 0.05$) showed follicles of this size. Cows in the G24 group had a smaller ($P < 0.05$) number of 12 to 16 mm follicles than the other groups. Conversely, those in the C group had a larger ($P < 0.05$) number of 7 to 11 mm follicles compared with the other groups ($P < 0.05$). At day 45, cows in all groups had similar proportions of follicles smaller than 11 mm, and similar quantities of follicles between 12 to 16 mm were found in the C and G48 groups. The G24 group had a larger proportion of these follicles ($P < 0.05$), while the G72 group had an intermediate number of follicles, similar to the other groups ($P > 0.05$). The G24 group had the lowest proportion of 17 to 21 mm follicles ($P < 0.05$).

The distribution of follicles diferred between days 25 and 45 in the C group with respect to structures smaller than 11 mm. The distribution of 12 to 16 mm follicles was similar ($P > 0.05$) in the two periods. In addition, a higher ($P < 0.05$) number of cows presented 17 to 21 mm follicles at day 45 when compared with day 25. For cows in the G24 group the distribution of follicles was similar in the category smaller than 11 mm. In contrast, the distribution was different at day 45, as more cows showed 12 to 16 mm follicles. Similar patters were observed in cows in the G48 and G72 groups at 25 and 45 days postpartum (Fig. 1).

The average back fat thickness (BFT) and body condition score (BCS) did not differ in the four experimental groups ($P > 0.05$). Back fat thickness at day 25 postpartum ranged from 3.8 ± 0.06 to 4.2 ± 0.8 mm, and at day 45 postpartum it ranged from 3.6 ± 1.0 to 4.2 ± 0.7 mm in all groups. The corresponding figures for BCS at day 25 ranged from 3.6 ± 0.9 to 4.4 ± 0.6, and for day 45, they ranged from 3.5 ± 0.7 to 4.2 ± 0.7 (Table 2).

Discussion

The unforeseen results of the pre-synchronization program somehow allowed us to compare the physiological responses in the two postpartum periods. The number of cows with estrus and ovulation at day 25 postpartum was not expected, and surpsingly, the average onset of estrus following CIDR withdrawal and the percentage of cows displaying estrus fell within the limits published for different essays [16].

The number of cows neither ovulating nor displaying estrus was

Table 2. Mean (± SEM) back fat thickness (mm) and body condition score at 25 and 45 days postpartum in the control group (C) and in the groups after being subjected to temporary weaning at 24 h (G24), 48 h (G48) and 72 h (G72)

	Average back fat thickness (mm)		Body condition	
Days postpartum	25	45	25	45
C	3.9 ± 0.3	4.2 ± 0.4	4.4 ± 0.3	4.2 ± 0.3
G24	4.1 ± 0.4	4.1 ± 0.5	3.9 ± 0.3	4.3 ± 0.3
G48	4.2 ± 0.4	4.0 ± 0.4	4.4 ± 0.3	3.5 ± 0.4
G72	4.2 ± 0.4	3.6 ± 0.4	3.7 ± 0.4	4.0 ± 0.3

different for day 25 in the control group (60%) compared with the other groups (10 to 20%). The values for day 45 were similar for all groups, ranging from 10 to 30%. In spite of the limitation of having a restricted sample, two interesting features are apparent in this set of data: the first is the poor response in the control group to the CIDR treatment at day 25 and the second is the acceptable reaction in all groups treated at day 45. To our knowledge, this is the first report of a synchronization regime plus calf separation so early after calving. Moreover, it renders information allowing to use the the first synchronization program as a successful priming to the most conventional breeding period at day 45 postpartum [8].

Another trait observed was the number of cows displaying estrus with no physiological support in a possible allelomimetic response, a phenomenon well documented in Zebu cattle following synchronization [17]. This characteristic was never found in more than 10% of the cows regardless of whether the cows had previously calved at 25 or 45 days. In contrast, a major feature was the number of cows ovulating but not displaying estrus, which amounted to around 20% of the cows. This result is in accordance with previous reports in Zebu cattle indicating that approximately one-fifth of cows do not exhibit overt signs of estrus [18–20]. In fact, Mukasamugerwa *et al*. [21] observed that 35.7% of heats were silent or missed visually following early weaning. In addition, Hoffman *et al*. [22] showed that cows with a calf present could ovulate but fail to exhibit overt signs of estrus.

In contrast, in the latter set of cows at day 45, the percentage of cows displaying estrus behavior and ovulation was higher in the G48 group and was similar among the other groups. Indirect evidence of these similarities could be explained by data of Pinheiro *et al*. [23], who concluded that the pregnancy rate of postpartum Nellore cows in acceptable body condition was not different when compared with that of cows treated with eCG or temporary calf separation and a synchronization protocol, including AI by appointment. This was also probably the case in our study, as no differences were found in body condition or back fat even from day 25 postpartum. Correa-Orozco *et al*. [24] measured back fat thickness and body condition at different reproductive stages and concluded that the score of the latter was correlated with the former only in nonpregnant cows. In a recent study, Mulliniks *et al*. [25] found that variations in body condition score in cows calving for the first time were not a factor for sound reproductive performance. In accordance with this, Lents *et al*. [26] established that duration and number of mounts received at the first estrus postpartum were not influenced by BCS or the supplement given. Whatever the case might be, it is obvious that by using a pre-synchronization program, the possibility of more cows becoming pregnant and achieving a calving interval close to the 12–13 month range increase. More research is needed to establish the importance of nutrition at the time of an early synchronization program with a fast uterus recuperation during the early postpartum.

Concerning follicular activity, our results confirm previous findings indicating that Zebu cattle could start cycling before 50 days postpartum [4, 6]. This early cyclicity does occur irrespective of the time allocated for separation of the calves from their dams. Martins *et al*. [9] found that repeating five 48-h calf withdrawals with 20-day intervals resulted in a greater cow pregnancy rate with a reduced period of postpartum anestrus.

However, the effect of calf separation can be masked by the BCS of the animals. Bishop *et al*. [27] found that the frequency of LH pulses, serum IGF-1 and the interval to onset of ovarian activity were influenced by body condition at weaning but not by day after weaning. This finding could explain the early restoration of ovarian activity in the present report, none of the groups differred in BCS or back fat measurements. Nonetheless, treatments including calf separation require long-term studies; Quintans *et al*. [28] found that the number of days from calf separation to conception was similar in cows temporary weaned for 96 to 144 h. Moreover, calf removal, particularly in combination with GnRH treatment, can induce a high proportion of beef cows to ovulate, but restoration of estrous cycles may not be achieved.

The proportion of follicles in the different categories was distinctively different between the controls and cows separated from their calves for 24 h when compared with those separated from their calves for 48 or 72 h. As expected, animals with shorter or no calf separation had a different pattern of follicular development, with apparent inhibition of the ability of intermediate follicles (12–16 mm) to become apparent as units of a bigger size (17–21 mm). Sa Filho *et al*. [29] showed a positive correlation between follicular size and fertility. Are cows separated from their calves for only 24 h or not at all capable of developing a dominant follicle that will lead to gestation ? This question remains to be answered. Based on our studies, the number of cows capable of ovulating (hence at risk of becoming pregnant) in these early stages of the postpartum period seems remarkable. An obvious limitation in our study is the lack of proper follow-up of the first ovulation at day 25 to discern if the estrous cycles were of normal duration.

The average BFT and BCS in the four experimental groups in the two periods were not different. Cows with a higher proportion of 17 to 21 mm follicles., BFT values above 3.5 mm and with

regular body condition, were significantly different regardless if they remained with their calves or were separated, and the length of this separation, thus demonstrating that physiologically animals are capable of becoming pregnant at least under the conditions of the present study.

Hence it can de concluded that (1) a pre-synchronization program at day 25 could trigger the onset of ovarian activity and facilitate a breeding program at day 50 and (2) temporary weaning enhances the effect of pre-synchronization program.

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