—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

Libia PÉREZ-TORRES¹⁾, Ivette RUBIO²⁾, Manuel CORRO²⁾, Abraham COHEN³⁾, Agustín ORIHUELA¹⁾, Carlos S. GALINA³⁾ and J. Luis PABLOS⁴⁾

¹⁾Facultad de Ciencias Agropecuarias, Universidad Autónoma del Estado de Morelos, Morelos 62210, México

²⁾Centro de Enseñanza Investigación y Extensión en Ganadería Tropical, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Veracruz, México

³⁾Departamento de Reproducción, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México 04510 México

⁴⁾Departamento de Genética y Bioestadística, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México 04510 México

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

(J. Reprod. Dev. 61: 199–203, 2015)

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.

Received: September 24, 2014

Accepted: January 29, 2015

Published online in J-STAGE: February 28, 2015

©2015 by the Society for Reproduction and Development

Correspondence: A Orihuela (e-mail: aorihuela@uaem.mx)

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

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 20° 4'N and 97° 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 identifying 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, however, 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 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)

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 differed 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 patterns 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 surprisingly, 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 ($\pm$ 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)

Days postpartum	Average back fat thickness (mm)		Body condition	
	25	45	25	45
C	3.9 $\pm$ 0.3	4.2 $\pm$ 0.4	4.4 $\pm$ 0.3	4.2 $\pm$ 0.3
G24	4.1 $\pm$ 0.4	4.1 $\pm$ 0.5	3.9 $\pm$ 0.3	4.3 $\pm$ 0.3
G48	4.2 $\pm$ 0.4	4.0 $\pm$ 0.4	4.4 $\pm$ 0.3	3.5 $\pm$ 0.4
G72	4.2 $\pm$ 0.4	3.6 $\pm$ 0.4	3.7 $\pm$ 0.4	4.0 $\pm$ 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 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, Mukasamugerva *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 differed 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 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 pre-synchronization program.

Acknowledgment

The authors are grateful to the PAPITT IN200107 program for financial support of this project. Thanks are given to Pfizer Mexico (presently Zoetis Mexico) for donation of the hormonal material utilized in the study. This study is part of an MSc thesis (Libia PÉREZ-TORRES) supported by the Masters Scholarship program (CoNaCyT México).

References

- Murillo Medina AL, Córdova-Izquierdo A, Soriano Robles R, Mendoza Martínez GD, Castillo-Juárez H. Breed differences in calving interval in the humid Mexican tropic. *Trop Anim Health Prod* 2009; **41**: 1357–1362. [Medline] [CrossRef]
- Williams GL. Suckling as a regulator of postpartum rebreeding in cattle: a review. *J Anim Sci* 1990; **68**: 831–852. [Medline]
- Montiel F, Ahuja C. Body condition and suckling as factors influencing the duration of postpartum anestrus in cattle: a review. *Anim Reprod Sci* 2005; **85**: 1–26. [Medline] [CrossRef]
- Rubio I, Castillo E, Soto R, Alarcón F, Murcia C, Galina CS. Postpartum follicular development in Brahman cows under two stocking rates. *Trop Anim Health Prod* 2010; **42**: 539–545. [Medline] [CrossRef]
- Molina R, Galina CS, Maquiver M, Estrada S, Chávez A, Díaz GS. Pregnancy rate in zebu cows with two different postpartum intervals exposed to a two-bull rotational system. *Vet Res Commun* 2003; **27**: 671–680. [Medline] [CrossRef]
- Henao G, Olivera-Angel M, Maldonado-Estrada JG. Follicular dynamics during postpartum anestrus and the first estrous cycle in suckled or non-suckled Brahman (*Bos indicus*) cows. *Anim Reprod Sci* 2000; **63**: 127–136. [Medline] [CrossRef]
- Galindo J. Effect of changes in body weight, body condition and back fat during last month of pregnancy on the reproductive efficiency of *Bos indicus* cows in the tropics of Costa Rica. *Open J Vet Med* 2013; **03**: 22–28. [CrossRef]
- Enriquez DH, Ungerfeld R, Quintans G, Guidoni AL, Hotzel MJ. The effects of alternative weaning methods on behaviour in beef calves. *Livest Sci* 2010; **128**: 20–27. [CrossRef]
- Martins PGMA, Arthington JD, Cooke RF, Lamb CG, Araujo DB, Torres CAA, Guimaraes JD, Mancio AB. Evaluation of beef cow and calf separation systems to improve reproductive performance of first-calf cows. *Livest Sci* 2012; **150**: 74–79. [CrossRef]
- Galina CS, Rubio I, Basurto H, Orihuela A. Consequences of different suckling systems for reproductive activity and productivity of cattle in tropical conditions. *Appl Anim Behav Sci* 2001; **72**: 255–262. [Medline] [CrossRef]
- Pérez-Torres L, Orihuela A, Corro M, Rubio I, Cohen A, Galina CS. Maternal protective behavior of zebu type cattle (*Bos indicus*) and its association with temperament. *J Anim Sci* 2014; **92**: 4694–4700. [Medline] [CrossRef]
- Pulido A, Zarco L, Galina CS, Murcia C, Flores G, Posadas E. Progesterone metabolism during storage of blood samples from Gyr cattle: effects of anticoagulant, time and temperature of incubation. *Theriogenology* 1991; **35**: 965–975. [Medline] [CrossRef]
- Schröder UJ, Staufenbiel R. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J Dairy Sci* 2006; **89**: 1–14. [Medline] [CrossRef]
- Wagner JJ, Lusby KS, Oltjen JW, Rakestraw J, Wettemann RP, Walters LE. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy requirement during winter. *J Anim Sci* 1988; **66**: 603–612. [Medline]
- Altman DG. Practical Statistics for Medical Research. 1st ed. Washington, D.C. Chapman and Hall: 1997.
- Galina CS, Orihuela A. The detection of estrus in cattle raised under tropical conditions: what we know and what we need to know. *Horm Behav* 2007; **52**: 32–38. [Medline] [CrossRef]
- Forster K, Galina CS, Maquiver M, Van der Laan G, Arnoni R, Verduzco A. Assessment of the intensity of behavioural traits and ovulation between synchronized and non-synchronized cows. *Reprod Domest Anim* 2007; **42**: 566–570. [Medline] [CrossRef]
- Bó GA, Baruselli PS, Martine MF. Pattern and manipulation of follicular development in *Bos indicus* cattle. *Anim Reprod Sci* 2003; **78**: 307–326. [Medline] [CrossRef]
- Bolaños J, Galina CS, Estrada S, Forsberg M. Resumption of postpartum ovarian activity monitored by plasma progesterone in anoestrous zebu (*Bos indicus*) cattle following temporary weaning and progestogen treatment. *Reprod Domest Anim* 1997; **32**: 267–271. [CrossRef]
- Vaca LA, Galina C, Fernández-Baca S, Escobar J, Ramírez B. Progesterone levels and relationship with the diagnosis of a corpus luteum by rectal palpation during the estrous cycle in Zebu cows. *Theriogenology* 1983; **20**: 67–76. [Medline] [CrossRef]
- Mukasa-Mugerwa E, Tegegne A, Franceschini R. Influence of suckling and continuous cow-calf association on the resumption of post-partum ovarian function in *Bos indicus* cows monitored by plasma progesterone profiles. *Reprod Nutr Dev* 1991; **31**: 241–247. [Medline] [CrossRef]
- Hoffman DP, Stevenson JS, Minton JE. Restricting calf presence without suckling compared with weaning prolongs postpartum anovulation in beef cattle. *J Anim Sci* 1996; **74**: 190–198. [Medline]
- Pinhoiro VG, Souza AF, Pegorer MF, Satrapa RA, Ereno RL, Trinca LA, Barros CM. Effects of temporary calf removal and eCG on pregnancy rates to timed-insemination in progesterone-treated postpartum Nellore cows. *Theriogenology* 2009; **71**: 519–524. [Medline] [CrossRef]
- Correa-Orozco A, Pulgarin-Velasquez F, Mesa H, Uribe-Velasquez F. Relationship between body condition and rump fat thickness in Zebu cows with different reproductive states. *Rev Cient-Fac Cien Vet* 2013; **23**: 42–47.
- Mulliniks JT, Cox SH, Kemp ME, Endecott RL, Waterman RC, Vanleeuwen DM, Petersen MK. Relationship between body condition score at calving and reproductive performance in young postpartum cows grazing native range. *J Anim Sci* 2012; **90**: 2811–2817. [Medline] [CrossRef]
- Lents CA, White FJ, Ciccioli NH, Wettemann RP, Spicer LJ, Lalman DL. Effects of body condition score at parturition and postpartum protein supplementation on estrous behavior and size of the dominant follicle in beef cows. *J Anim Sci* 2008; **86**: 2549–2556. [Medline] [CrossRef]
- Bishop DK, Wettemann RP, Spicer LJ. Body energy reserves influence the onset of luteal activity after early weaning of beef cows. *J Anim Sci* 1994; **72**: 2703–2708. [Medline]
- Quintans G, Viñoles C, Sinclair KD. Follicular growth and ovulation in postpartum beef cows following calf removal and GnRH treatment. *Anim Reprod Sci* 2004; **80**: 5–14. [Medline] [CrossRef]
- Sá Filho MF, Crespilho AM, Santos JEP, Perry GA, Baruselli PS. Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled *Bos indicus* cows. *Anim Reprod Sci* 2010; **120**: 23–30. [Medline] [CrossRef]