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Effect of alternating bulls as a management tool to improve the reproductive performance of suckled Zebu cows in the humid tropics of Costa Rica

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Abstract

A study was undertaken to evaluate the effect of alternating bulls between a single and a multiple sire mating (MSM) program on the reproductive performance of suckled Zebu cows raised under range conditions in the humid tropics of Costa Rica. Multiparous Zebu cows ($n = 94$) suckling calves were distributed between two experimental trials (A and B) consisting of 47 animals each. A single sire mating (SSM) system was alternated weekly with a MSM system with three bulls. This period lasted for 8 weeks. To facilitate estrous expression, four cows were strategically synchronized (estrus-stimulated) in alternate weeks. Courtship predominated over mounting under non-stimulated estrus, for each mounting performed an average of 6.0 and 6.3 courtship activities were recorded in the SSM and MSM, respectively. Under the influence of strategic synchronization corresponding values were 3.9 and 4.2 in the SSM and MSM, respectively ($P > 0.05$). Blood samples for progesterone evaluation were taken twice weekly. All cows in trial A were in anestrus at the start of the study. By second week, 5 out of the 47 cows had initiated estrous cycles and by the third week six were pregnant. In contrast in trial B, 9 out of 47 had initiated estrous cycles before interacting with the bulls and on week 3, only two females had become pregnant and three had initiated estrous cycles. Significant differences were found in the cumulative percentage of cows pregnant between trials A and B ($P < 0.05$). Even though these results occurred, the rotation of the bulls

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(one or three), or the type of cows (estrus-stimulated or not) did not influence the results in this study.

Keywords: Zebu bulls; Mating systems; Body condition; Estrous cycles; Progesterone; Anestrus

1. Introduction

In tropical regions natural breeding using one or several bulls is still the most frequent method making the selection of males one of the most important procedures in any cattle enterprise. Nonetheless, studies on reproduction of Zebu bulls (*Bos indicus*) under grazing conditions are rare (Galina and Arthur, 1991). Furthermore, the long postpartum interval to resumption of ovarian activity is another of the main obstacles to improving productive efficiency of cattle in the tropics. Studies in the USA have shown that bulls can have a potential benefit in reducing the postpartum interval in *Bos taurus* cattle (Burns and Spitzer, 1992; Stumpf et al., 1992; Hornbuckle et al., 1995) and this benefit has been shown in Zebu herds (Bolaños et al., 1997, 1998).

In the traditional systems of breeding, problems such as aggression, dominance and interference among bulls affects the reproductive performance of the herd (Blockey, 1979; Fields et al., 1982). To minimize these effects, it is necessary to introduce different methods of sire mating. To this effect, a periodic rotation of the bulls during the mating period might be a strategy to improve reproductive performance in the herd, thus, potentially increasing the biostimulatory effect. In addition, if a reduced group of cows are to be used as a method for strategic control of the estrous cycle, it is hoped that both interventions might stimulate the formation of groups of cows that are sexually active thus shortening the mating period.

The objectives of the present study were (1) to alternate a single and a multiple sire mating (MSM) system in anestrous-suckling cows, (2) to measure the effect of strategic estrous synchronization as a possible management tool to increase reproductive efficiency, (3) to monitor ovarian activity and pregnancy in postpartum cows following bull exposure, (4) to assess the effect of body condition on herd reproductive performance.

2. Materials and methods

The present study was conducted in the Bovine Production Unit of the Agronomy School of the Technological Institute of Costa Rica, located in San Carlos, Alajuela (10°25′N latitude 84° longitude 32 W). This area is situated 75 m above sea level in the humid tropics. Mean pluvial precipitation is 3096 mm with and average temperature of 24 °C and a relative humidity of 85.3%.

Multiparous Zebu cows ($n = 94$) suckling calves were used. Animals were managed under pasture conditions in a paddock containing Pará (*B. mutica*), Native grass (*A. compressus*/*P. notatum*), African Star grass (*C. plectostachyus*) Ratana grass (*I. indicum*) and Kudzú (*P. phaseoloides*) during the rainy season. Animals were distributed in two experimental trials consisting of 47 females. The first trial started on 9 June and ended on 4 August. The second started 4 August and concluded on 29 September. The body weight of the cows was 452 ± 11 kg for trial A and 404 ± 73 kg for trial B. Body condition score (BCS), measured on a scale of 1–5 points (Pullan, 1978), averaged 2.9 ± 0.3 for trial A and 2.7 ± 0.4 for trial B. Animals were assigned considering those with an average of BCS above 2.5 throughout the study as opposed to those below this figure. At the start of the bull(s) exposure, the cows of trial A were 53 ± 9 days postpartum and all were in anestrous, according to progesterone (P_4) levels in blood obtained twice weekly. Cows from trial B were 66 ± 15 days postpartum and 81% of them were anestrus. Brahman bulls ($n = 4$) aged between 4 and 6 years and with a mean body weight of 708 ± 32 kg were used. Previously, the males underwent breeding soundness evaluation to verify that their reproductive ability was satisfactory.

A mounting period was established for 16 weeks subdivided in two phases of 8 weeks each. First, a single sire mating (SSM) system was employed followed by a multiple sine mating (MSM) system with three bulls. The sequence of the above-mentioned bull(s) exposure systems is indicated in the following scheme:

Week	Breeding	Bull(s)
Trial A		
1	Single	A
2	Multiple	A, B, C
3	Single	D[a]
4	Multiple	D, B, C[a]
5	Single	C
6	Multiple	C, D, A
7	Single	B[a]
8	Multiple	B, D, A[a]
Trial B		
9	Single	D
10	Multiple	D, B, C
11	Single	A[a]
12	Multiple	A, B, C[a]
13	Single	B
14	Multiple	B, D, A
15	Simple	C[a]
16	Multiple	C, D, A[a]

[a] Four animals synchronized to facilitate a possible biostimulatory effect (estrus-stimulated).

Synchronized animals received an ear implant consisting of 6 mg of *Norgestomet* along with an intramuscular injection of 3 mg of *Norgestomet* and 5 mg of *Estradiol Valerate* (Synchromate-B; Merial, Mexico). The implant was removed after 9 days coinciding with the start of the respective bull(s) exposure period.

The sexual behavior of the animals during the bull(s) exposure, such as smelling and licking the genital area, butting, supporting the head over a female, and the sign of flehmen (courtship activities) were observed and recorded. Mounting and mounting attempts were classified as mounting activities according to the procedure described by Orihuela et al. (1983). Observations were performed twice a day (06:00–08:00; 16:00–18:00) until a total of 320 h were completed. The rotation of bulls applied as a management tool was also used to compare the quality and type of activities. This comparison was undertaken taking into consideration the number of bulls present at the time and the possible influence of the cows induced to estrus as a biostimulatory tool.

Blood samples were collected twice weekly by venipuncture to determine the changes on progesterone levels and to define the reproductive status of each cow during the study. Progesterone values higher than 2.9 nmol/l. were taken as an indicator of the presence of ovarian activity (Bolaños and Molina, 1992). When two consecutive values above 2.9 nmol/l were obtained the animal was considered to have initiated estrous cycles. Five consecutive elevations of P_4 were indicative of pregnancy.

The evolution of the reproductive status of each one of the trials was established from the individual levels of P_4. Thirty-five days after the end of the bull(s) exposure period, rectal palpation for pregnancy established the ability of the bulls to impregnate the females. An analysis of variance was used to compare the sexual activities of the bulls; also descriptive statistics (average and standard deviation) were used in the analysis of the sexual data of the bulls and of the reproductive performance of the cows. A logarithmic lineal model was used to compare the difference between trials with respect to body condition and pregnancy rate. A Chi-square was used to discern possible differences within trials, between animals with an average above 2.5 in BCS and those below this figure. In order to analyze the cumulative percentage of pregnant cows in the two trials a Log rank test was used to compare the survival curves.

2.1. Definitions

1. Average of courtship actions conducted by the bulls, per sexually active cow (CB/cow), was calculated by dividing the total number of courtship activities of the bulls by the number of sexually active cows that interacted with them.
2. Average mounting performed by the bulls, per sexually active cow (MB/cow) resulted from dividing the total number of mountings performed by the bulls by the number of sexually active cows that interacted with them.
3. Ratio of the number of pregnancies in relation to the number of cows that had initiated estrous cycles (PC/cow) is the consequence of dividing the total number of pregnant cows by the number of cows initiating estrous cycles during the experiment.

3. Results

3.1. Herd sexual activities

Table 1 contains the data for mounting and courtship activities performed by one bull (SSM) or the combination of three bulls (MSM) in groups where cows were stimulated and non-stimulated to induce estrus (one or three bulls present). Courtship behavior (CB)

Table 1
Mating behavior performed by Zebu bulls according to the type of breeding employed, as well as to the type of estrus stimulation treatment applied

Week	Bull	Number of cows	Courtship behavior	Relation: CB/cow[a]	Mounting behavior	Relation: MB/cow[b]
Single sire mating system						
1	A	16	49	3.1	8	0.5
5	C	11	32	2.9	9	0.8
9	D	18	88	4.9	21	1.2
13	B	16	60	3.8	0	0
–	Subtotal	61	229	–	38	–
3	D[c]	23	135	5.9	38	1.7
7	B[c]	16	86	5.4	34	2.1
11	A[c]	20	102	5.1	6	0.3
15	C[c]	16	94	5.9	29	1.8
–	Subtotal	75	417	–	107	–
Multiple sire mating system						
2	ABC	38	218	5.7	14	0.4
6	ACD	22	66	3.0	7	0.3
10	BCD	31	179	5.8	34	1.1
14	ABD	25	107	4.3	36	1.4
–	Subtotal	116	570	–	91	–
4	BCD[c]	23	128	5.6	41	1.8
8	ABD[c]	33	147	4.5	6	0.2
12	ABC[c]	33	144	4.4	43	1.3
16	ACD[c]	34	203	6.0	58	1.7
–	Subtotal	123	622	–	148	–

[a] CB/cow is calculated by dividing the total number of courtship behavioral activities of the bulls by the number of sexually active cows that interacted with them.
[b] MB/cow results from dividing the total number of mounting behavior activities performed by the bulls by the number of sexually active cows that interacted with them.
[c] Estrus-stimulated period.

predominated over mounting behavior (MB). Under non-stimulated estrus for each mount performed a total of 6.0 and 6.3 CB were recorded in the SSM and MSM, respectively. Under the influence of stimulated estrus corresponding values were 3.9 and 4.2 in the SSM and MSM, respectively.

Table 2 contains data for mean amount of courtship performed by the bull(s) in relation to the number of sexually active cows (CB/cow). These figures were 4.6 ± 1.2 in the SSM and 4.9 ± 1.0 in the MSM. MB per sexually active cow (MB/cow) was 1.0 ± 0.8 in the SSM and 1.0 ± 0.6 in the MSM. In both cases there were no statistically significant differences ($P > 0.05$).

When a bull or bulls were confronted with cows in the non-stimulated estrus group (Table 2) CB per sexually active cow (CB/cow) was 4.2 ± 1.2, whereas under the influence of stimulated estrus, corresponding values were 5.3 ± 0.6 ($P < 0.02$). In contrast, MB per sexually active cow (MB/cow) was 0.7 ± 0.5 in the non-stimulated estrus group and 1.4 ± 0.7 in the stimulated estrus group with averages being non-significant ($P > 0.05$).

Table 2
Relationship between the quantity of courtship and mounting behaviors performed by the bulls and the number of sexually active cows, according either to the type of breeding method, as well as to the type of estrus[a]

Effect	Active cows	Courtship behavior	Relation: CB/cow[b]	Mounting behavior	Relation: MB/cow[c]
Breeding					
Simple	17.0 ± 3.5	80.8 ± 32.7	4.6 ± 1.2 a	18.1 ± 14.3	1.0 ± 0.8 a
Multiple	29.9 ± 5.8	149.0 ± 5.8	4.9 ± 1.0 a	29.9 ± 18.8	1.0 ± 0.6 a
Estrus					
Non-stimulated	22.1 ± 8.9	99.9 ± 65.8	4.2 ± 1.2 a	16.1 ± 13.1	0.7 ± 0.5 a
Stimulated	24.7 ± 7.6	129.9 ± 37.5	5.3 ± 0.6 b	31.9 ± 18.0	1.4 ± 0.7 a

[a] Values with different letter within the same column indicates significant differences ($P < 0.05$).

[b] CB/cow is calculated by dividing the total number of courtship behavioral activities of the bulls by the number of sexually active cows that interacted with them.

[c] MB/cow results from dividing the total number of mounting behavior activities performed by the bulls by the number of sexually active cows that interacted with them.

Fig. 1 depicts that during both SSM and MSM, the most frequent CB was smelling and licking the genital area (SLG), along with flehmen (FLH). In the case of MB, the most frequent activity was mounting attempt (MA). During the MSM, the display of agonistic behavior was as low as 3% (45/1476) of the total number of behavioral activities recorded. Aggressive butting behavior (71%), threatening 16% and chasing 13% were the actions most frequently observed.

3.2. Herd reproductive performance

Changes in progesterone levels throughout the study are depicted in Figs. 2 and 3. Progesterone patterns indicated that for trial A 9% (4/47) of cows were in anestrus during the period (Fig. 2) whereas in trial B the percentage increased to 30% (14/47), possibly affecting reproductive performance (Fig. 3).

Different patterns of ovarian activity between trials were obtained prior to and during the breeding period. In trial A, even though 100% of cows were anestrus at the start of the study, by week 2 (18 June), 5 out of the 47 cows had initiated estrous cycles; and during the third week (21 June) six were pregnant. In contrast, 9 out of 47 animals in trial B had initiated estrous cycles before interacting with the bull(s) and by week 3 (13 August), only two females had became pregnant and only three had initiated estrous cycles. In trial A, five animals initiated estrous cycles at some stage during the study but failed to continue a typical pattern of estrous cycles or resumed estrous cycles after an abnormally long period of transition between the cycles (example, cows 1114, 246, 3290, 482 and 817). In contrast, in trial B there were eight cows with such irregularities (130, 258, 345, 386, 404, 409, 466, 621). Whereas in trial A, only 4 of 47 cows were anestrus at the end of the study, the corresponding figure for trial B was much greater 14/47 ($P < 0.05$).

At the beginning of each period of bull(s) exposure, the number of cows that had initiated estrous cycles and anestrus were similar in both trials. However, differences in the evolution of these two categories together with the numbers of cows initiating estrous cycles and that

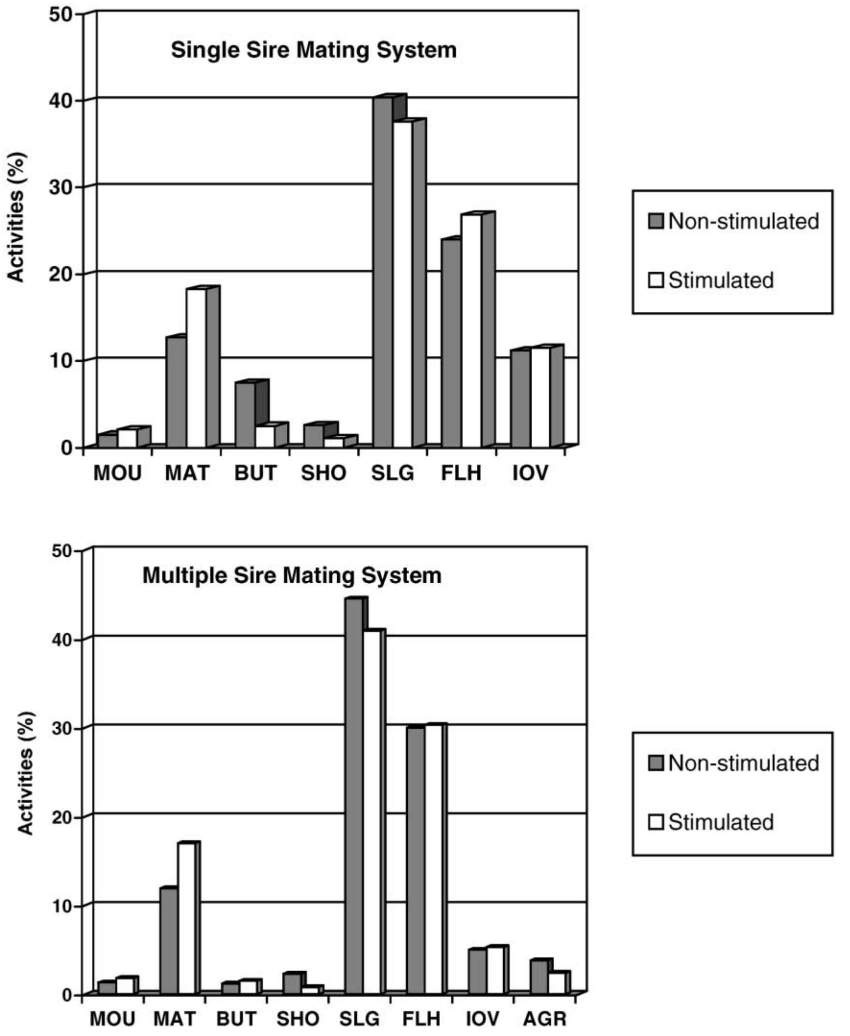

Fig. 1. Type of breeding behavior performed by Zebu bulls under the influence of non-stimulated estrus and stimulated estrus. MOU: mount; MAT: mount attempt; BUT: attraction butting; SHO: supporting head over another animal; SLG: sniff and/or lick genital area; FLH: flehmen; IOV: interest over a certain female; AGR: aggressions.

were pregnant were observed in subsequent weeks (Fig. 4) for example, whereas in trial A the number of anestrous cows decreased to less than 20% by week 4, in trial B the percentage never decreased to below 50%. This disparity was reflected in pregnancy rate throughout the study, when compared trial A and B. In trial A the pregnancy rate 72% (34/47) whereas in trial B this figure was 40% (19/47) ($P < 0.05$). The mean pregnancy rate achieved in both trials was 56.4% (53/94). The cumulative pregnancy per week (Fig. 5) shows that during the first 4 weeks of bull(s) exposure in the trial A, 27 cows became pregnant (79% of the total

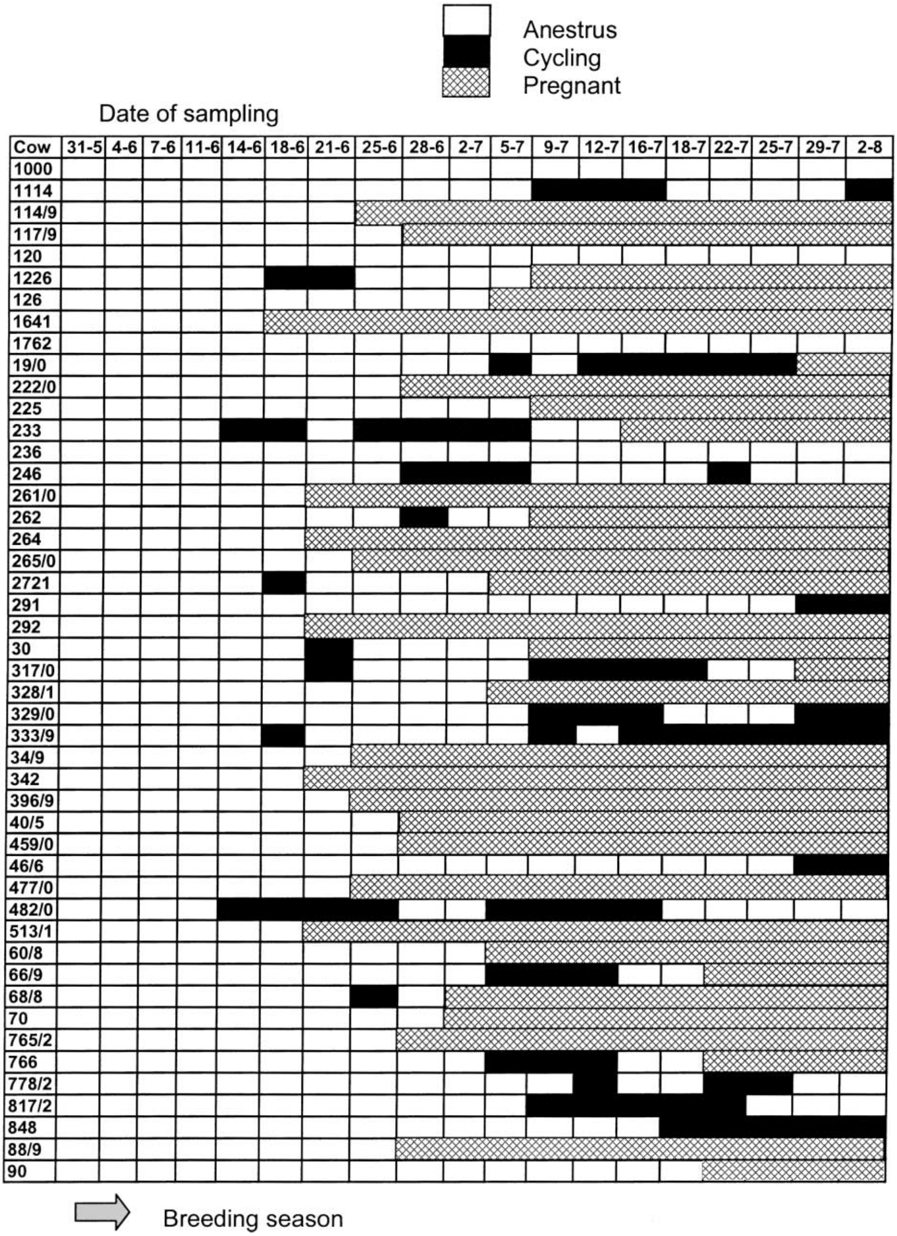

Fig. 2. Pattern of progesterone in Zebu cattle exposed to a natural breeding system.

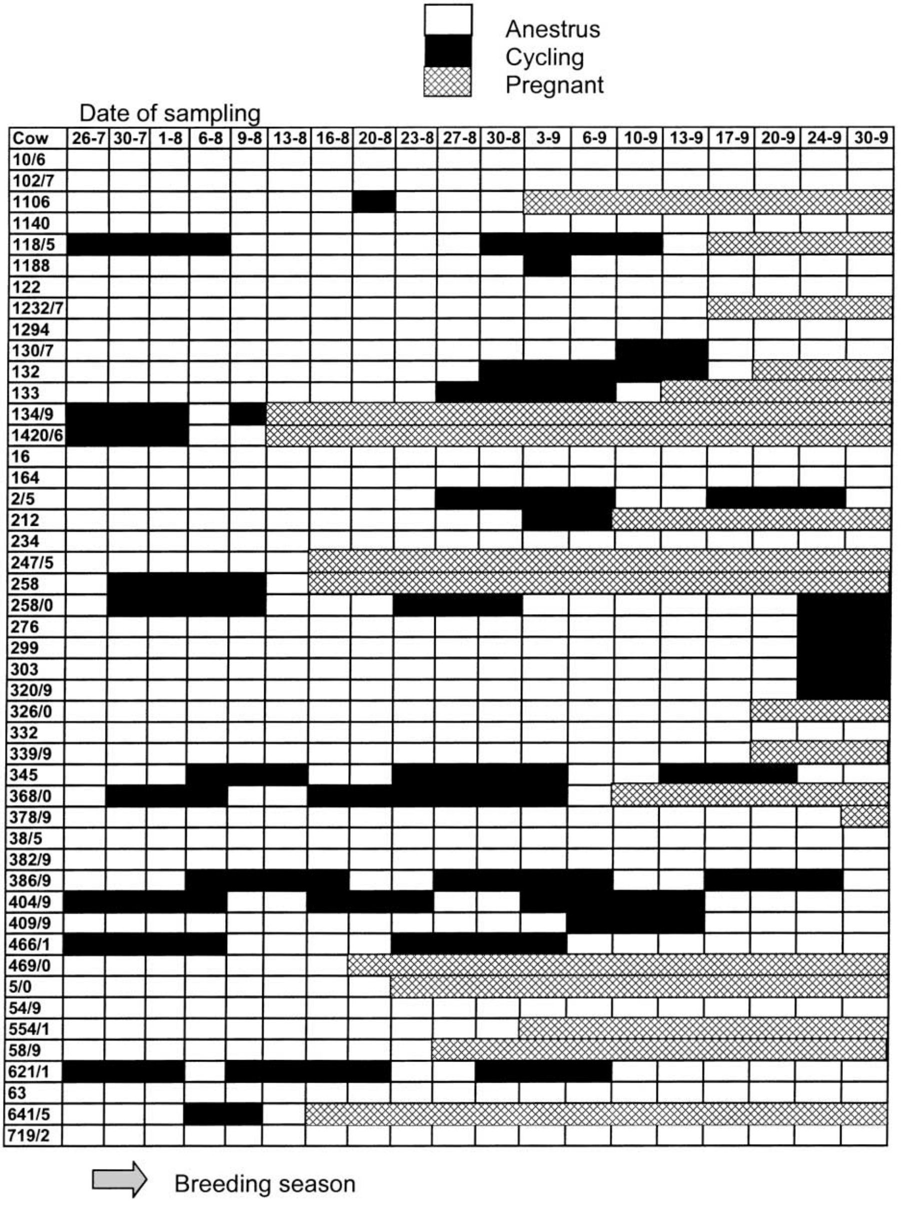

Fig. 3. Pattern of progesterone in Zebu cattle exposed to a natural breeding system.

of pregnant females). In the following 4 weeks, seven more cows became pregnant (21% of the total). This contrasts with the results for trial B, where there were only 10 pregnant cows (53% of the total) in the first 4 weeks and nine more cows (47% of the total) in the last four.

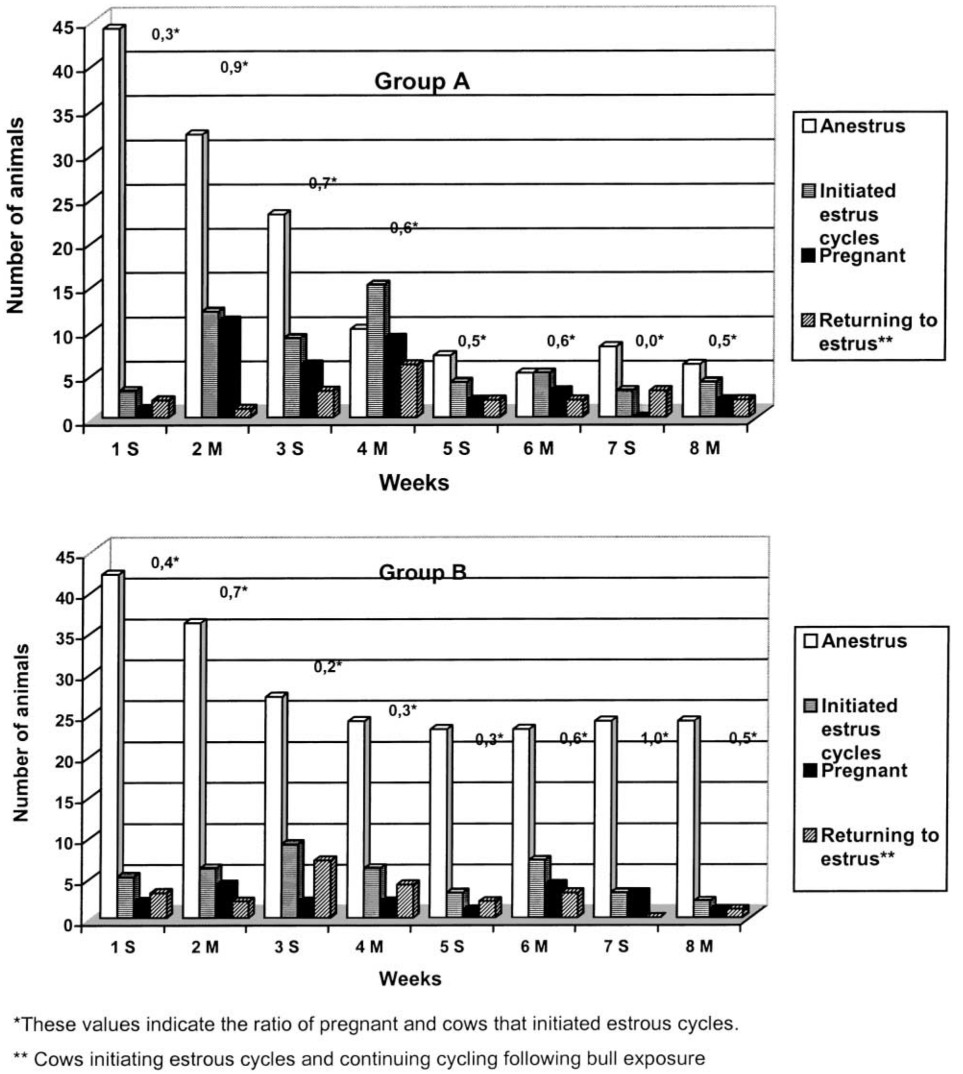

Fig. 4. Reproductive categories of the cows according to the two breeding periods in the two experimental trials (Groups A and B). Single asterisk (*) indicate the ratio of pregnant and cows that initiated estrous cycles; double asterisks (**) indicates cows initiating estrous cycles and continuing cycling following bull exposure.

Table 3 shows the weekly averages in the number of cows with progesterone levels indicative of initiation of estrous cycles. This figure was 4.9 $\pm$ 2.6 in the SSM and 7.1 $\pm$ 4.4 in the MSM ($P > 0.05$). This same relationship was seen with respect to the type of estrous treatment (non-stimulated estrus or stimulated estrus, 5.6 $\pm$ 2.9 versus 6.4 $\pm$ 4.4, respectively). Furthermore, there were no differences in the number of pregnant cows independent of the type of bull(s) exposure system (single or multiple), or the type of

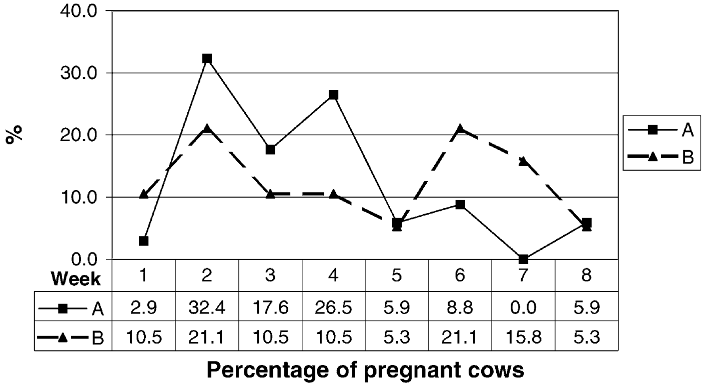

Fig. 5. Comparison of the cumulative percentages of pregnant cows during the two breeding periods in the two experimental trials (Groups A and B).

Table 3
Weekly average of cows that initiated estrus cycles and pregnant cows during the experimental period, according to the breeding method and type of estrus[a]

Effect	Cows initiating estrous cycles	Pregnant cows	Ratio (GC/cow)[b]
Breeding			
Single	4.9 ± 2.6 a	2.1 ± 1.8 a	0.43 ± 0.3 a
Multiple	7.1 ± 4.4 a	4.5 ± 3.6 a	0.59 ± 0.2 a
Estrus			
Non-stimulated	5.6 ± 2.9 a	3.5 ± 3.2 a	0.54 ± 0.2 a
Stimulated	6.4 ± 4.4 a	3.1 ± 2.9 a	0.48 ± 0.3 a

[a] Values with the same letter in the same column indicate no significant differences ($P < 0.05$).
[b] GC/cow is the consequence of dividing the total number of pregnant cows by the number of cows cycling during the experiment.

estrus. Also, the relationship between the numbers of cows initiating estrous cycles that became pregnant (GC/cow), was not significant considering the different bull(s) exposure system and/or the type of estrous stimulation treatment with approximately 50% of females becoming pregnant of the total of animals initiating estrous cycles.

Table 4
Number of pregnant and non-pregnant cows related to body condition score (BCS)

	Trial A			Trial B		
BCS	>2.5	≤2.5	Total	>2.5	≤2.5	Total
Pregnant	29[a]	5	34	16	3[c]	19
Non-pregnant	8[b]	5	13	16	12[d]	28
Total	37	10	47	32	15	47

[a] and [b] differ significantly $P < 0.08$.
[c] and [d] differ significantly $P < 0.05$.
[a] and [c] differ significantly $P < 0.02$.

Table 4 contains data for variation in the body score condition indicating that in group A 15% (5/34) of the pregnant cows had an average BCS below 2.5 whereas in group B this value was 16% (3/19). In the case of the cows non-pregnant in group A, 38% (5/13) had an average BCS below 2.5, while in group B 43% (12/28) ($P < 0.01$), however in the case of group B, the difference between cows with BCS above 2.5 and those below in pregnancy was significant ($P < 0.05$) and this tended to be significant for group A ($P < 0.07$).

4. Discussion

The behavior of the bulls showed similar patterns under the presence of one or three bulls and the type of estrous stimulation treatment evaluated as bulls consistently performed more courtship (80–86%) than MB. This is in agreement with previous studies (Molina et al., 2000) where it was demonstrated that Zebu bulls showed between 75 and 86% more courtship than MB. It has been observed that bulls spend an important amount of time identifying cows in estrus, and similarly attempting to mount females in anestrus (Orihuela et al., 1988; Rodríguez et al., 1993). Studies in *B. indicus* males have shown a low frequency of MB (Chenoweth and Osborne, 1975; Landivar et al., 1985 and Price, 1987). A critical aspect in the evaluation of the reproductive behavior of Zebu bulls could be that the performance of the male is established according to the receptivity of the female. It is possible that at certain times, bulls show limited mounting activity due to the anestrous condition of the females and as a consequence waste energy interacting with cows that will not come into estrus in the short term. In effect, earlier studies have shown that Zebu bulls spend less time mounting cows as compared with bulls of European breeding (Larsen et al., 1990).

Costa e Silva et al. (1993) concluded that pregnancies with Nelore cattle in Brazil were not affected by the ratio of bulls to cows but rather was influenced by the individual variation among bulls. Similarly, Silva-Mena and Delgado-Leon (2000) found no relation between libido score and pregnancy rate working with Zebu cattle in Mexico. Under the conditions employed in the present study, no differences in the number of pregnant cows at the end of the study were established, neither as a consequence of the combination in number of bulls (one or three), nor of the type of estrus evaluated (stimulated versus non-stimulated). This agrees with previous research (Molina et al., 2000) where it was shown that the presence of several bulls in a breeding group is not necessary to achieve a pregnancy rate similar to that expected when one bull is present. This conclusion holds at least when the ratio of bulls to females in a multiple sire system is about 1:15. In effect, Godfrey and Lunstra (1989) working with crossbred European bulls found that males with high and low serving capacity achieved similar levels of mating activity when placed in a SSM system. In contrast, confronted in a double sire mating system, only bulls classified as having a high serving capacity will serve more females. Moreover, Farin et al. (1982) found that the number of mounts per bull was not different in a single sire from a MSM even after estrous synchronization. Furthermore, in accord with the present study, the aggressive activities among bulls were kept to a minimum, as the availability of cows in estrus was high. Obviously more research is needed on the applicability of these methods of breeding, such as experiments where the bull to female ratio is higher to force males to express their true reproductive potential. Mikeska and Williams (1988) Neville et al. (1988) and Williams (1988) achieved a more desirable response in the induction of estrous cycles and fertility following synchronization and bull presence. Our results do not confirm these findings although the method of synchronization did not include the whole herd.

Poor body condition has been shown to counteract any favorable effects of postpartum interventions Bolaños et al. (1997) in accord, cows in higher body condition do not benefit from the effect of bull biostimulation when compared with cows in moderate condition (Stumpt et al., 1992). As the monitoring of BCS in the short term is difficult to conduct, Holness et al. (1980) variations among studies are to be expected. In effect, the performances of the cows in the two trials and particularly the ones that became pregnant were quite different (Figs. 2 and 3).

The final pregnancy rate obtained in the present study (56%) is similar to numerous reports in the tropics suggesting that only 50% of the total herd will be calving per year (Voh and Otchere, 1989; Silva-Mena and Delgado-Leon, 2000). However, it is disturbing that following close progesterone monitoring, only 50% of the cows that had initiated estrous cyclicity became pregnant. This finding agrees with previous research undertaken by this group. In effect, Galicia et al. (1999) determined that of the 10 cows that had initiated estrous cycles as indicated by progesterone concentrations, only five or less became pregnant after mating with a bull. The discrepancy between the percentage of cows with a possibility of becoming pregnant and the percentage remaining non-pregnant at the end of the breeding period deserves further investigation. Others have indicated the possibility of embryo death as a factor (Gregory et al., 1996; Holroyd et al., 1979). The 14% of cows initiating estrous cycles and showing erratic progesterone patterns coupled with an estimated embryonic death rate of 20% might explain why most of the cows failed to sustain pregnancy after exposure to the bull. Also, postpartum progesterone patterns can be short and inconsistent after bull exposure leading to poor fertility (Alberio et al., 1987; Hornbuckle et al., 1995). More studies are obviously needed, with larger number of animals per treatment, to determine the relative roles of the males and of the females in this reproductive failure.

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