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Behavioral Characteristics of *Bos indicus* Cattle after a Superovulatory Treatment Compared to Cows Synchronized for Estrus

A. Jiménez, F. Bautista, C. S. Galina, J. J. Romero[1,*] and I. Rubio[2]

Department of Animal Reproduction and Artificial Insemination,
Faculty of Veterinary Medicine and Animal Science, National Autonomous University of Mexico, Mexico

ABSTRACT : The intensity and duration of sexual behavior in *Bos indicus* was assessed through the continuous observation of sexual receptivity. Two groups of cows were formed: only synchronized (n = 50) and other group further superovulated (n = 20). An intravaginal implant that released progesterone over 9 d was used. After removing the implant, 25 mg of $PGF_{2\alpha}$ was administered. In the superovulated group, the administration of 280 mg (Follicle stimulant hormone) FSH-P1 per cow with a decreasing dosage over 4 d was utilized. In both groups, behavioral observations began at the moment of implant removal. Sexual behavior was analyzed using a Kruskal-Wallis test to compare the mean of hours in estrus, effective mountings and number of mounts/hour during estrus. A nonparametric survival analysis was performed using the time in two ways: i) when an event happened it was placed in a 24 h time-frame and, ii) the time of observation in continuous form (96 h) assessing the difference between curves by the log rank test Chi-square. The only significant difference was the number of mounts/h during receptivity ($p<0.05$). In the superovulated group three periods of sexual activity during the day were identified, with these events being of greater frequency and duration than the synchronized group ($p = 0.02$); besides, the superovulated group began estrus before the synchronized group ($p = 0.0035$) when using the total period. In a simulation study, when the number of observations went from two (06:00-18:00) to three periods (06:00, 12:00 and 18:00) cows detected accurately (<6 h after the onset) increased more than 20%. The results show that superovulated cows presented greater intensity and duration of sexual activity in contrast with only synchronized animals. (**Key Words :** Sexual Behavior, *Bos indicus*, Superovulation, Synchronization, Estrus)

INTRODUCTION

The low intensity and short duration of signs of estrus in *Bos indicus* indicates that the work needed to correctly detect this period in cows is difficult and imprecise (Bo et al., 2003; Galina and Orihuela, 2007). It has been speculated that one of the reasons why the female Zebu presents weak signs of estrus is because the follicular diameter is generally smaller than in *Bos taurus* (Bo et al., 2003). It is likely that a direct relationship exists between the follicular diameter and the quantity of estrogens that are synthesized by the theca interna (Bridges and Fortune, 2003), and this possibly affects the intensity of the signs of sexual receptivity (Van Eerdenburg et al., 2002). In female *Bos taurus*, it has been shown that a positive correlation exists between the concentration of estrogens in the follicle and the intensity of the signs of estrus (Lyimo et al., 2000).

Based on the quantity of follicular liquid present in the ovaries, females with extreme follicular growth enhanced by superovulation treatments should present more intense sexual activity when compared with females having only one ovulatory follicle. The objective of this study was to measure the intensity and to quantify standing events exhibited by continuously observing sexual behavior according to the method proposed by Orihuela et al. (1983).

MATERIALS AND METHODS

Location

The experiment was carried out in the F1-Heifer Production Unit "La Soledad", located on Km 3.5 of

* Corresponding Author : J. J. Romero. Tel: +506-22602155, Fax: +506-22602155 E-mail: jromero@medvet.una.ac.cr
[1] School of Veterinary Medicine, Universidad Nacional, Heredia, Costa Rica.
[2] Centre for Education, Research and Extension in Tropical Animal Husbandry Faculty of Veterinary Medicine and Animal Science, National Autonomous University of Mexico, Tlapacoyan, Veracruz, Mexico.
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highway Martínez de la Torre-Novara, Municipality of Atzalán, Veracruz, Mexico (19°50′ N, 97°1′ W). The annual average temperature is 25.2°C with an average annual rainfall of 1,679 mm and an elevation of 150 m.a.s.l. The climate has been classified as Af(m)w″(e) hot and humid (Garcia, 1973) with rains all year, and without a defined dry period. The farm belongs to the Centro de Enseñanza, Investigación y Extensión en Ganadería Tropical (CEIEGT), Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México.

Experimental animals

Bos indicus cows were allocated to one of two groups, one formed with 50 synchronized cows, while the second comprised 20 synchronized and superovulated females. All animals were multiparous, non-pregnant, and non-lactating cows, and were maintained in rotationally grazed pastures. The cows had an average body condition of 3.0 (2.5 to 3.5) on a scale of 1 to 5, where 1 represents an emaciated cow and 5 an obese animal, the females were between 6 and 8 years of age, and the average weight was 494±57.68 kg. There was no difference in the percentage of animals cycling previous to the treatment, being 50.0% in synchronized and 60% in the superovulated cows ($p = 0.60$, x^2)

Synchronization

Both groups were synchronized using an intravaginal progesterone releasing device (Eazy-breed CIDR™, Pfizer®, Mexico), that stayed *in situ* for 9 d. At the moment the implants were removed, an intramuscular injection of 25 mg of prostaglandin F2α (Lutalyse™, Pfizer®, Mexico) was administered.

Multiple ovulation

Total dose per donor was 240 mg of follicle stimulating hormone (Folltropin®-V, Bioniche Canada) administered in decreasing dose during days 9, 10, 11, and 12 of the estrous cycle. In the afternoon of day 11, and morning of day 12, cows received an intramuscular injection of 25 mg of prostaglandin PGF2α (Pfizer, México).

Detection of sexual receptivity

The time at which sexual receptivity began in the superovulated and the synchronized groups was determined by continuously observing sexual receptivity over 96 h once the implant was withdrawn. All standing events and the time at which each event was performed were recorded. To obtain this information, two observers were assigned for periods of 3 h each (Orihuela et al., 1983). Each activity was subdivided into sexual behaviors (e.g. mounting and intention of mounting) and courtship (Eerdenburg et al., 1996; Maquivar et al., 2006), including head bumping, flehmen, sniffing, following, licking and assisted head resting (Galina et al., 1982; Orihuela et al., 1988). The length of estrus was considered to occur when three mountings happened within 1 h, with the period bounded before and after by a time span of three or more hours of inactivity (Galina et al., 1982).

Serum progesterone determination

The serum progesterone (P4) levels were used to determine the ovarian activity with two samples taken 3 d apart on average 15 d prior to treatment. Further samples were taken in order to evaluate the effect post treatment; blood samples were obtained at the 7th and 14th after the average presence of estrus in the experimental groups. The samples were collected by coccygeal venipuncture using Vacutainer® tubes and needles. The P4 serum determination was carried out by radioimmunoassay at the Laboratory of Endocrinology of the Universidad Nacional Autónoma de México (UNAM). Samples with >1 ng/ml P4 in two occasions were classified as with an active corpus luteum.

Statistical analysis

The overall proportion of animals showing estrus was calculated, as well as the number and variations of standing events in the superovulated and synchronized groups. To determine the effect of the treatments on sexual behaviors, a Kruskal-Wallis test was performed to compare the average length of estrus, number of effective mountings, and number of mountings/h during sexual receptivity. Additionally, a non-parametric survival analysis by Kaplan-Meier curves was performed on the time spent in each activity using the time in two forms: i) the time at which an event happened was placed in a 24 h time-frame to determine the existence of periods of greater intensity, and ii) the time of observation in continuous form was analyzed (96 h) to determine the instant span of the events and to observe at what time there was a greater occurrence of cases, establishing whether the curves were different or not using the log rank Chi-square test.

Based on the results of continuous observation, a simulation study was undertaken to assess the precision of detecting the onset of estrus. The points in time were compared if observations were carried out twice (06:00 and 18:00 h) or adding a third point (12:00 h). These points in time were chosen taking in consideration previous experiences on the diurnal peaks of estrual activity (Orihuela and Galina, 2007). To analyze the data, three arbitrary periods were chosen: animals detected 0 to 3 h from the onset of estrus, those between 4 and 6 h and a third group of animals detected with more than 6 h from the onset of estrus. The global percentage of animals detected in estrus in each scheme was calculated, coupled with the accuracy in detecting them in the periods chosen. A third

Table 1. Comparison of percentages of cows cycling before and ovulation after the synchronization and superovulation treatments

Treatment group (n)	Cycling		Ovulation		Post-treatment P4 levels (n)	
	n	%	n	%	Low	High
Synchronized (50)	25	50[a]	46	92[a]	6	44
Superovulated (20)	12	60[b]	15	75[a]	4	16

The frequency of cows with high and low levels of progesterone post-treatment is also presented.
Different letters represent statistically significant differences ($p<0.05$) between treatments.

Table 2. Comparison of means for the time spent in sexual receptivity, number of effective mountings, and the number of mountings per hour during estrus

Treatment / Groups (n)	Variable	Mean	SD	Median	Kruskal-Wallis test (H)*
Synchronized (50)	Length of estrus	11.96 [a]	8.64	13.50	2.88
	Complete mountings	30.96[a]	29.48	22.50	0.10
	Mountings/hour during estrus	1.94[a]	1.62	1.55	6.48
Superovulated (20)	Length of estrus	8.76[a]	6.53	7.00	
	Complete mountings	27.05[a]	28.14	13.00	
	Mountings/hour during estrus	2.84b[b]	1.55	2.50	

SD = Standard deviation. Different letters represent statistically significant differences ($p<0.05$).
* Indicates the comparison of the variable between synchronized and superovulated.

calculation was based on the percentage of animals detected within 6 h after the onset of estrus.

RESULTS

The overall percentage of cows that ovulated after the treatments was 87.1%, with no statistical difference between groups, being 92,0% for synchronized, and 75.0% for superovulated ($p = 0.10$, x^2) (Table 1).

Comparing across sexual behaviors and courtships, superovulated animals conducted more mountings, mounting attempts, head bumping, flehmen, sniffing, following and licking, compared with the synchronized group (Figure 1), while the synchronized group participated more in assisted head resting.

The superovulated group showed approximately 50% more mountings/h during estrus compared to the synchronized group ($p<0.05$). No differences were observed between groups for the amount of time spent in sexual receptivity or in the percentage of complete mountings (Table 2).

In both treatments, there were three well identified periods of sexual activity: between 01:00 and 06:00, between 14:00 and 17:00 h, and between 21:00 and 23:00 h

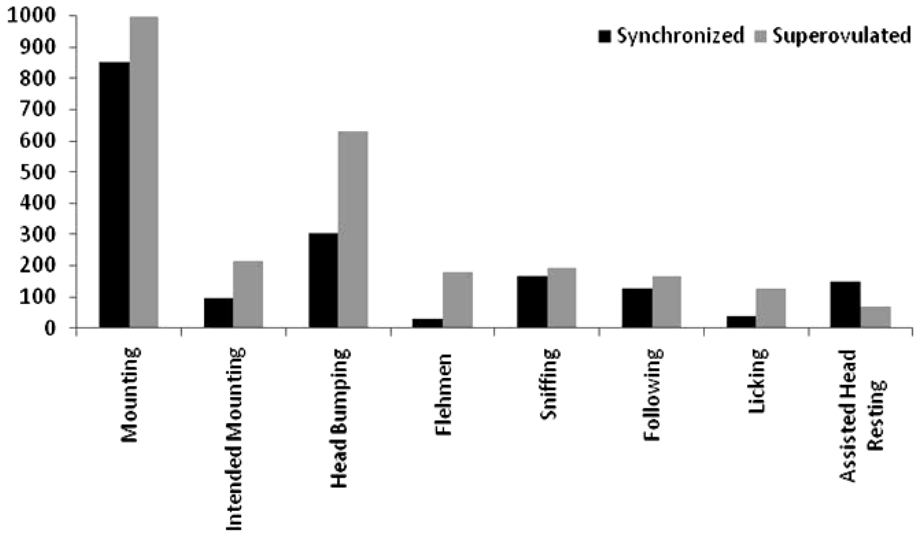

Figure 1. Frequency of sexual activity and courtship in the synchronized and superovulated groups.

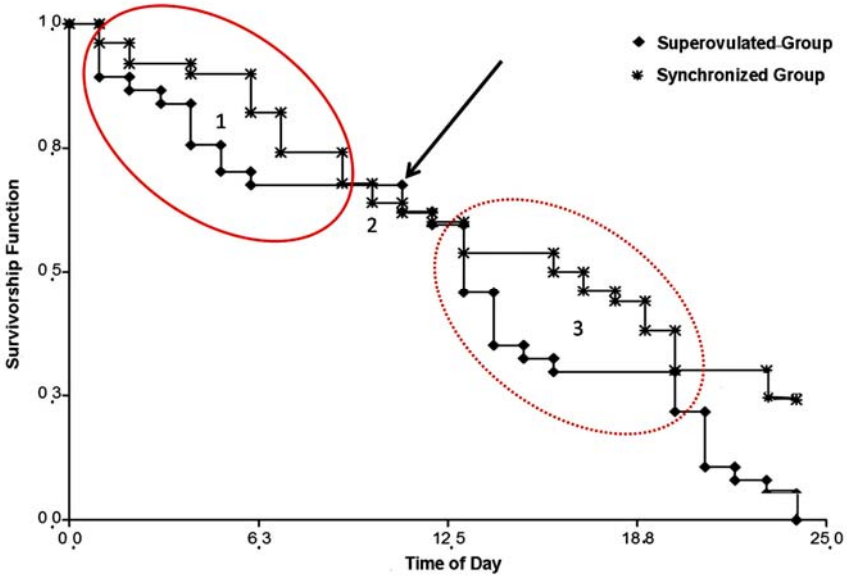

Figure 2. Kaplan-Meier curve on the estrous cycle of superovulated and synchronized in a 24 h period.

(Figure 2). In all periods, standing events were more frequent and happened earlier in the superovulated group (Logrank Test - Chi Square = 5.402, p = 0.020).

Over the 96 h period of observation since the removal of the implant in both groups, the superovulated group began estrus before the synchronized group (Log rank Test - Chi Square = 8.487, p = 0.0035). Two periods of estrus initiation were observed between 26 and 40 h (#1), and between 48 and 62 h (#2), with the latter period more prolonged and with a greater frequency of responses (more cows were sexually receptive) in the superovulated group (Figure 3).

Based on the results of continuous observation, Figure 4 and 5 depict the findings to assess the precision of estrus detection, if the observation for estrus was to be carried out during the three periods established. Figure 4 contains the global percentage of estrus which was 70.0% (35/50), the percentage of estrus detected from the total occurred corresponded to 91.4 (31/35), and the percentage of estrus detected within the 6 h from the onset of estrus which was 60.0% (21/35). If the observations are reduced to two (06:00 and 18:00 h) the percentages were 70.0, 85.7 and

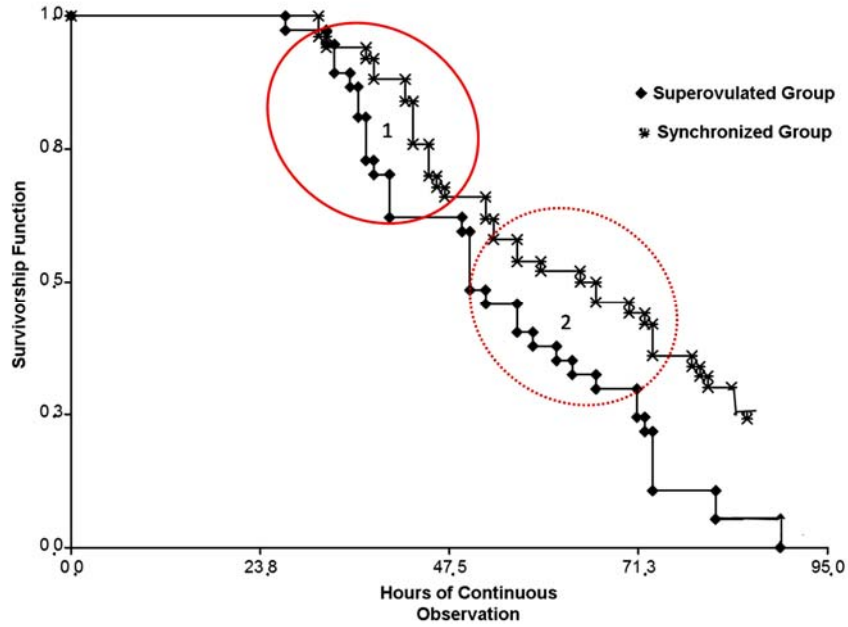

Figure 3. Kaplan-Meier curve on the estrous cycle of superovulated and synchronized. Hour 0 commences at implant removal and ends at 96 h.

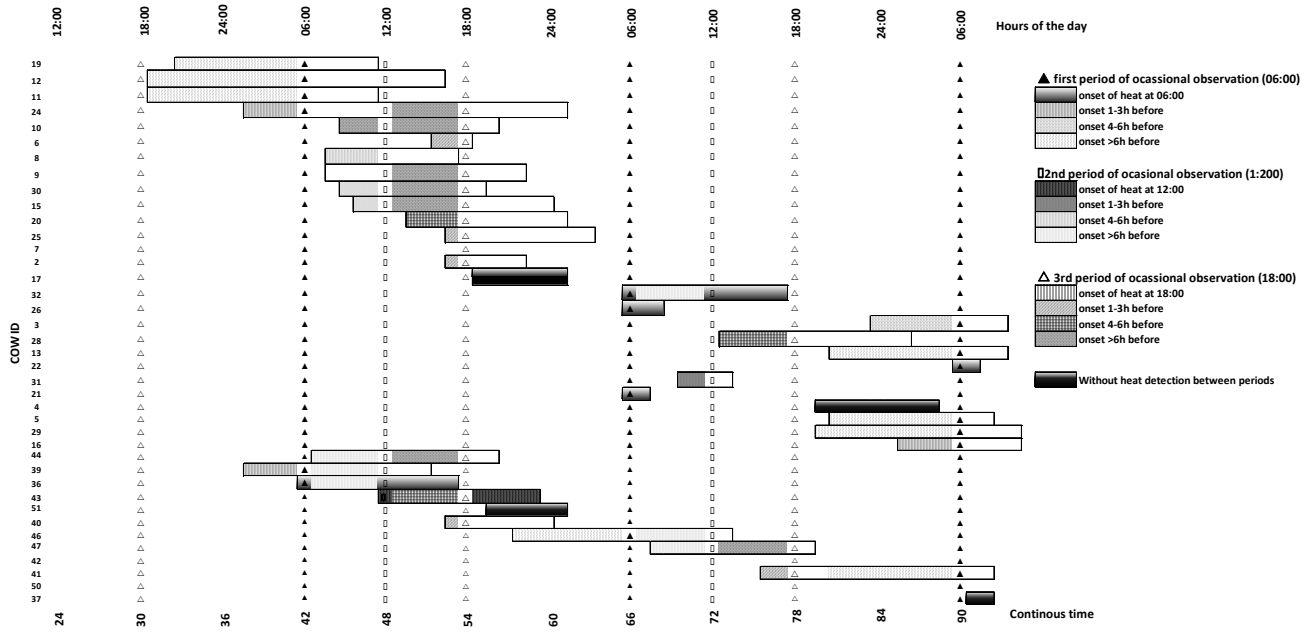

Figure 4. Comparison of three periods of observation (06:00, 12:00 and 18:00 h) in the synchronized cows following the results of continuous observation.

42.8, respectively. Figure 5 contains the percentage of estrus 78.9% (15/19) and the percentage of estrus detected from the total occurred which was 53.3 (8/15). Finally, the percentage of estrus detected within the 6 h from the onset of estrus corresponded to 53.3 (8/15). If the observations are reduced to two (06:00 and 18:00 h) the percentages were 78.9, 46.6 and 26.6, respectively. There were only significant differences in the percentage of estrus detected within the 6 h from the onset of estrus between two and three observations ($p<0.05$).

DISCUSSION

Responses to estrus, measured as the number of cows displaying estrus behavior, were similar between the synchronized and superovulated groups, and the results conform with the literature indicating an acceptable response to sexual receptivity as between 70 and 80% of the cows showing signs of acceptance to mounting (Galina and Orihuela, 2007). The intensity of this behavior was different between groups where the superovulated cows allowed more mountings, intended mountings, and generally more courting behavior, which should facilitate the detection of estrus. Based on quantifications of estrual behaviors it is possible to study the effect of diverse factors on the expression of sexual receptivity. One issue is the influence of the intensity and duration of estrus (Van Eerdenburg et al., 1996). The superovulated cows showed 46% more estrual activities per hour than the synchronized females at the expense of a shorter estrus periods. Intensity of sexual behavior can be driven by factors such as dominance, the social structure in the herd and the hormonal profile of the cows at the time of estrus (Galina and Orihuela, 2007). However, from the practical perspective, one can speculate that if estrus is more intense, this phenomenon should facilitate the expression of estrus in cows with weak signs.

Three periods of intensity in the signs of sexual receptivity existed in the groups (Figure 2). The first

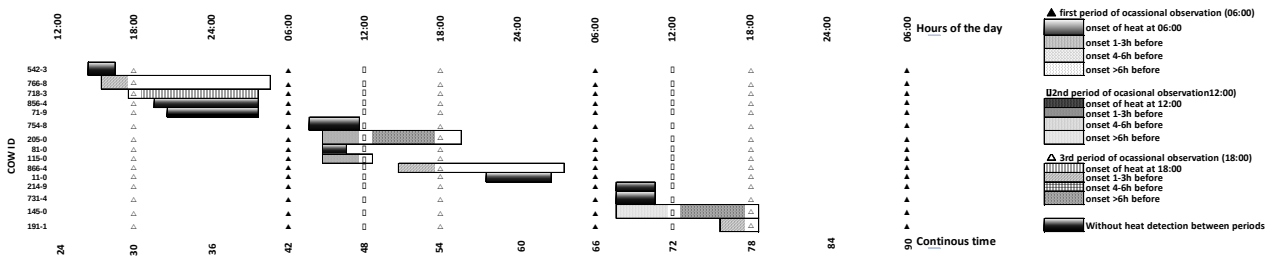

Figure 5. Comparison of three periods of observation (06:00, 12:00 and 18:00 h) in the superovulated cows following the results of continuous observation.

occurred very early in the morning (exactly before dawn), and conforms to previous reports indicating this to be a very intense period of mounting activity (Galina et al., 1982; Llewelyn et al., 1987; Mattoni and Ouedraogo, 2000); occurring first in the superovulated group possibly due to the intense follicular growth due to treatment with FSH (Fortune and Hansel, 1985). Likewise, although the non-superovulated cows showed more events, they were not with the same velocity as with the synchronized, which indicates a more solid distribution of standing events in the latter, facilitating, in theory the detection by the occasional observer. The second period of activity indicated by an arrow in Figure 2 denotes the similarity in both groups, as the curves almost concur at this point in time whereas by the time period three is reached, the separation of the groups is evidently apparent. The different pattern of expression of estrus in the two groups strongly suggests that the number of animals and the social dominance in the herd play an important role in the display of grouping estrus behavior (Johnson and Ono, 1986).

Unfortunately, it was not possible to obtain serial blood samples to ascertain the existence of differences in the concentration of estrogens in the two groups, nor it was feasible to compare the same number of animals in each group.

Responses to sexual receptivity after the moment the implant was removed showed patterns confirmed by other studies where two periods of sexual interaction can be observed, the first between 26 and 40 h, and the second between the 48 and 62 h. The first period usually is short and intense, while in the second the duration is increased because of a greater time span where the animals displayed estrus (Alonso et al., 2009).

The percentage of animals detected in estrus augmented as the number of observations increased. This finding is in accord to previous observations (Van Eerdenburg et al., 1996). Nonetheless, in the superovulated group, this advantage does not seem to be so apparent and is probably related to the small number of animals. Insignificant participants in a sexually active group diminish the possibility of intense signs of estrus. In effect, Williamson et al. (1972) demonstrated a drastic reduction in the number of mounts if two or three animals were in estrus concomitantly. The fact that the cows in the superovulatory group responded with greater intensity at the expense of less hours in estrus, reinforces the concept that these animals are quite possibly under a heavy influence of estrogens. In conclusion, the group treated with FSH had greater follicular development and greater concentrations of estradiol, a hormone that modulates sexual behavior, leading to more activity (Lymio et al., 2000). However, factors inherent in behavior (Galina and Orihuela, 2007) also play important roles in the demonstration of sexual behavior and may have a greater influence than physiological factors, suggesting further investigation is warranted.

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