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Average Response to Estrus and Timing of Ovulation in *Bos indicus* Cattle Synchronized Alternatively with a Synthetic Progestagen

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Abstract: Eighteen multiparous Brahman cows were divided in three groups and synchronized consecutively with a nine-day interval between groups. After implant withdrawal, synchronized cows, together with those forming the sexually active group were subjected to intensive ultrasound exams to detect ovulation. Observation for signs of estrus was continuous and females were classified as a) showing overt signs, defined as those cows that mount and allowed mounting and b) active estrus as females that only mount. Some cows showed estrus with the groups they were synchronized and were classified as being concomitantly in estrus. The total percentage of cows in heat was 66%, of which 83% showed overt signs of estrus and the remaining 16% displayed active estrus. Eight cows were concomitantly in estrus, 75% of them in active estrus and 25% allowing and performing mounting behavior. The expected time of the onset of estrus after implant removal was different among groups ($p<0.0004$). Ovulation after implant withdrawal occurred in 83% of all animals, although some cows ovulated outside of the range of the group with which they were synchronized. The average time of ovulation after implant removal was similar ($p = 0.19$), for all groups. Differences were found in the dominant follicular size b etween animals showing overt signs (11.31±2.18 mm) and those displaying active estrus (7.33±1.80 mm) ($p<0.01$). All the synchronized females displaying overt signs of estrus ovulated contrary to those showing active estrus. Some cows ovulated without manifesting estrous behavior. It is concluded that cows need to display full estrus (mounting and allowed to be mounted) to be considered as candidates for ovulation and the system of synchronizing in alternation but having all the animals together, influences the number of females participating in the sexually active group as the experiment progresses.

Key words: Zebu cattle, estrous synchronization, estrous behavior, artificial insemination

INTRODUCTION

There has been an increase in the use of Artificial Insemination (AI) in cows under semi-intensive conditions in the tropics during recent years. However, the widespread implementation of AI has been impeded by ineffective and inaccurate estrus detection, a prerequisite for proper timing of AI. Numerous cows are inseminated when they are not in full estrus, partly due to the lack of adequate observation of estrous signs by the producers. The beginning of estrus is the most commonly used predictor to time the event of ovulation; generally artificial insemination is programmed from the moment of the onset to 18-24 h afterwards[1]. The duration of estrus in *Bos indicus* cattle is significantly shorter (11 h) than *Bos taurus* (18 h), making the detection of the actual onset difficult under field conditions[2]. Likewise, there is great variability in the onset of estrous behavior and the average time of ovulation when utilizing an estrus synchronization scheme. Pinheiro *et al.*[2] reported that this event occurred 26 h after the onset of estrus. In contrast, Cavalieri *et al.*[3] and Barros *et al.*[4] indicated that

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ovulation takes place 150 h after progestagen implant withdrawal, independently of the onset of estrus. Maquivar *et al.*[5] and Solano *et al.*[6] evaluated the onset of estrus after synchronizing animals in alternation and found that some cows could show sexual activity without the presence of a dominant follicle, hence complicating the interpretation of the timing from the onset of overt estrous signs, the presence of a follicle and the time of ovulation.

In a recent review Orihuela[7] proposes that the social structure in a herd may hinder the display of estrous behavior. In effect, it has been observed that cows not ovulating, show imitational sexual behavior with other cattle especially if they are not all synchronized at the same time[8]. Therefore, it is possible that cows might be detected in estrus and inseminated when they have no possibility of becoming pregnant. For this reason, the objectives of this study were to determine the occurrence of estrus and ovulation in synchronized Zebu cows and to establish the relationships between an alternating synchronization program and the behavioral interaction among synchronized and non-synchronized cows.

MATERIALS AND METHODS

Location: The present study was carried out at the Center for Teaching, Research and Extension in Tropical Animal Husbandry belonging to the Faculty of Veterinary Medicine, National Autonomous University of Mexico, located in the State of Veracruz, Mexico at 20°4'N y 97°3'O and classified as tropical humid climate[9].

Animals and treatment: Eighteen cyclic, non-suckled multiparous Brahman cows were monitored and grouped in 3 groups (6 cows per treatment). All of them were synchronized consecutively, with Synchromate B (SMB, Merial, Mexico). SMB treatment consisted of an ear implant containing 6 mg of norgestomet, an active progestagen, plus a 2 mL intramuscular injection containing 3 mg of norgestomet and 5 mg of estradiol valerate in sesame oil on the day of implant insertion. The implants were removed 9 days after insertion. Each group was synchronized with a difference of 9 days between treatments. This was applied to create periods when different numbers of cows were expressing estrus at the same time and to observe the interactions between synchronized and non-synchronized animals. All three groups of cows were maintained in the same paddock during the experimental period.

Sexual behavior: Sexual activities were registered following implant withdrawal in the three experimental groups according to the method proposed by Orihuela *et al.*[10]. All animals were painted with large numbers the flanks to ensure their identification. The animals were observed continuously for 100 h after implant withdrawal. The beginning of estrus was defined as when three or more mounts were received or performed by a cow with not more than 3 h between each mount.

Behavioral signs of estrus were classified according to the following criteria:

- Showing overt signs, defined as those cows that mounted and/or allowed mounts by others.
- Active estrus. Cows performing mounting behavior but not allowing themselves to be ridden by others.

A cow was only classified in one category at a given time and those animals showing estrous behavior either by mounting or allowing to be mounted, or by only receiving mounts outside the synchronized groups, were designated asynchronously in estrus.

The expected time for expression of estrous behavior after implant withdrawal was from 24 to 72 h. Ovulation was expected to occur between 26 to 30 h after the onset of estrus.

Reproductive evaluation: Ovarian activity was monitored in all cows by ultrasonography every two days for the length of the experiment. However, 24 h after implant withdrawal, cows were subjected to an intensive period of ultrasonographic observations (every 6 h for 240 h) with the objective of determining the moment of ovulation, which was defined as the disappearance of the largest follicle = 8 mm of diameter followed by confirmation of an active corpus luteum capable of producing progesterone[11,12]. Blood samples were taken every two days for the duration of the trial. Progesterone concentrations were used to determine ovarian activity. The samples were handled based on the recommendations of Pulido *et al.*[13] and centrifuged at 1500 rpm for 10 min.

The plasma were analyzed using a solid phase radioimmunoassay in 100 μL of serum using commercial kits (Pharmaceuticals, Diagnostic Division). The intra and interassay coefficients of variation were 7.41 and 6.18%, respectively. When the concentration of progesterone was $\geq$1 ng mL^{-1} in 2 or more continuous samples, the animal was considered to have a functional corpus luteum.

Statistical analysis: A logistic regression was performed to determine the probability that an animal in a given group will display estrus. Differences in the time to estrus and ovulation were determined by survival analysis,

where non-respondents were given a right censor value of 100 h if not observed and 240 h if ovulation was not detected by ultrasound. The Chi-square test was used to analyze of the number of cows ovulating and the relation between type of estrus and ovulation rate among the three experimental groups. The duration and onset of estrus following implant withdrawal and size of the preovulatory follicle were analyzed by GLM, SAS[14,15]

RESULTS

Total percentage of cows in estrus after implant withdrawal (within 100 h) was 66% (12/18) for the three experimental groups. From those, 83 % of cows showed overt signs of estrus and the remaining 16% displayed active estrus. The probability of an animal to come in estrus was 50% (3/6) for the first and second groups in order of synchronization and 100% (6/6) for the third group synchronized ($p<0.01$). There were 8 cows that displayed sexual activity asynchronously, i.e., not together with their respective treatment group. Of these, 75% were in active estrus and 25% allowing and performing mounting behavior. The type of estrus displayed in each group is indicated in Fig. 1-3. The expected time of the onset of estrus after implant withdrawal was different ($p<0.0001$) among the three groups, with median values of 76.2, 74 and 38 h for the first, second and third groups, respectively. The duration of estrus in the three groups did not vary significantly (9.8±1.9 for A, 10.5±2.6 B and 7.4±1.9 h C) ($p = 0.12$), nor did the time of estrus in synchronized cows 8.7±2.3 h and in the asynchronous group 8.8±4.8 h ($p = 0.95$).

A total of 83% (15/18) of the cows ovulated after implant withdrawal. The median time of ovulation after implant removal was similar ($p = 0.12$), 150, 101.5 and 65.5 h respectevely, for the three groups. Just one of the animals ovulated outside of their groups in which they were synchronized (Fig. 1-3). In every experimental group 5/6 of the females ovulated, within the first group treated 40% (2/5) displayed overt signs of estrus, 20% (1/5) active estrus and the rest did not show heat. In the group treated second, 60% (3/5) showed overt signs of estrus and 40% did not show heat. In the third group, 5 of the 6 females displayed overt signs of estrus.

Figure 1 to 3 depict the ovarian activity in the three groups judged by ultrasound and blood progesterone concentrations prior to and after implant withdrawal, together with the average of the observations in the intensive ultrasound period to detect the time of ovulation. All the synchronized females displaying overt signs of estrus ovulated (10/18). Two cows showed only active estrus of which one ovulated outside the expected time and the other did not ovulate. The remaining six females did not showed estrous behavior, four of them ovulated without displaying overt signs of estrus and the other two did not ovulate. Ninety three percent of synchronized and concomitant cows showing overt signs ovulated, contrary to those only displaying active estrus ($p = 0.012$). Differences ($p<0.01$) were found in follicular size between animals showing overt signs (11.31±2.18 mm; $n = 16$) and those displaying active estrus (7.33±1.80 mm; $n = 9$). In contrast, no differences were observed ($p>0.05$) between follicular diameter in the last exam prior to disappearance of the largest follicle in the

Fig. 1: Follicular development, moment when estrus was expressed following implant withdrawal and time of ovulation in synchronized and non-synchronized cows in the first synchronization

cows that ovulated following implant withdrawal for the three groups being 11.2±1.9, 12.4±1.9 and 13 ±2 mm, respectively.

DISCUSSION

The time of estrous presentation after implant withdrawal was shorter (38 h) and more cows (6/6) displayed behavioral estrus in the third synchronized group in relation to the other groups. It seems that animals go through a learning process and at the end of the experimental trial the females are more receptive to express full estrus. In previous experiments we have noticed the same trend[5,8,16]. Another explanation for this finding i s that by coincidence, fewer females (1/6) in the third group had a functional corpus luteum at the time of implant withdrawal as opposed to the other groups were 3/6 and 2/6 had a functional CL (Fig. 2 and 3). Based on this result it seems commendable to inject cows with a luteolytic agent at the moment of withdrawal[17]. In fact, six of the 18 cows synchronized had a functional CL judged by progesterone and all but one of the active cows had a CL present at the moment of implant withdrawal and when clinically displaying mounting behavior. Nonetheless,

Fig. 2: Follicular development, moment when estrus was expressed following implant withdrawal and time of ovulation in synchronized and non-synchronized cows in the second synchronization

Fig. 3: Follicular development, moment when estrus was expressed following implant withdrawal and time of ovulation in synchronized and non-synchronized cows in the third synchronization. (The figure was abbreviated 36 h due to technical difficulties)

there was no difference when overt signs of estrus were manifested after implant withdrawal and the time which this event lasted between synchronized and asynchronous animals (Fig. 1, 2 and 3).This finding is in agreement with the conclusions of Galina et al. [18], Porras *et al.*[19] and Umed *et al.*[20]. Estrus onset after implant withdrawal differed between groups, being more defined in the last synchronization, which could be the result of the effect of biostimulation previously mentioned, or to the fact that sexual behavior tends to be more compact when two or more animals are sexually receptive at the same time[8]. The average time of ovulation after implant withdrawal concurred with reports by Pinheiro *et al.*[2], Murray *et al.*[12] and Cavalieri *et al.*[3]. Four females in this experiment did not show overt signs and ovulated outside their expected time. This is in accord to other studies[10,21,22]; where cows did not express behavioral estrus despite a progesterone pattern indicative of cyclic activity. Furthermore, Johnson and Gambo[23], working with White Fulani heifers in Nigeria, found that only 9% of 32 heifers showed four consecutive heats during 112 days of continuous observation, thus indicating that all heifers showed silent estrus at least once or that the cycles were longer than expected. It is possible that these animals did not find a sexual partner or were unable to associate with other animals in similar physiological stages hence estrus was not fully expressed[11].

In addition, 44% of the animals showed concomitant estrus. It seems reasonable to assume that animals are capable of interaction with females in estrus imitating cows in this physiological event[5]. Studies in Zebu cattle have reported a marked synergism of estrous behavior particularly if progestagen synchronization is employed[11,24]. Wright *et al.* [25] found that the cervical mucous from cows in estrus produce pherohormones capable of inducing behavioral estrus in their peers. In the present study, of the 12 females exhibiting full estrus; 91% ovulated, suggesting that proper estrus, (i.e. that followed by ovulation) is characterized by the willingness of the cow to accept mounting[11]. This result disagrees with previous data in Zebu cattle, which indicated that 85% of the mounts (given or received) were performed by cows in estrus[10]. Our results also suggest that full estrus was the consequence of estrogen driven follicles as cows displaying estrus had larger follicles (11.31±2.18 mm), contrary to those only showing active estrus (7.33±1.80 mm). This result supports the concept that there is a correlation between estradiol concentration and estrous behavior[26]. Our results support the hypothesis that morphologically large follicles are good indicators of full estrus. It remains to be tested whether the size of a follicle determines the behavioral expression of estrus, as 50 % of the active cows could erroneously be declared in coming in estrus. However Van Eerdenburg *et al.*,[27] failed to find a correlation between follicular size and time of ovulation or estrous behavior score in Holstein cattle, suggesting that displaying estrus is a complex mechanism not entirely explained by follicular growth.

CONCLUSION

In the present study, all the synchronized females displaying overt signs of estrus ovulated, contrary to those only showing active estrus in which all but one displayed estrus activity with an active CL and large follicles (Fig. 1-3). Other cows synchronized ovulated without displaying behavioral estrus. It is concluded that displaying full overt signs (mounting and allowing mounting), contrary to only active estrus is a good predictor of ovulation and errors in detecting cows in estrus are to be expected when small numbers of animals are synchronized.

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