ABSTRACT: Five experiments were conducted on commercial farms in Brazil designed to develop the basis for an estrus synchronization protocol using melengestrol acetate (MGA) in *Bos indicus* cattle. These studies resulted in the development of the following protocol: 0.5 mg·d⁻¹ of MGA between d −14 and −1; 2.0 mg i.m. injection of estradiol cypionate on d −9; 48 h temporary weaning between d 0 and 2; and natural service beginning on d 0. The basis of this protocol was to induce estrous cyclicity before postpartum loss of body condition, prevent premature luteolysis, eliminate the need for labor required to detect estrus, and consequently increase the likelihood of pregnancy early during the postpartum period. This treatment effectively induced estrous cyclicity among anestrous cows, synchronized estrus activity, and prevented premature luteolysis with no negative effect on pregnancy.

Key words: anestrous, *Bos indicus*, estrous synchronization, melengestrol acetate

INTRODUCTION

*Bos indicus* cattle have longer lengths of gestation and postpartum anestrus (Abeygunawardena and Dematawewa, 2004), making it difficult to adhere to a 365-d calving interval (Yavas and Walton, 2000; Bó et al., 2003; Baruselli et al., 2004). Cows losing BCS, in the presence of a calf, or both, experience reduced frequency LH pulses (Williams et al., 1983; Imakawa et al., 1987) in a manner that dominant follicles fail to reach ovulatory size and secrete sufficient estradiol to induce an LH surge (Wiltbank et al., 2002). Because BCS often decreases as number of days postpartum increases (Meneghetti and Vasconcelos, 2008), strategies to induce estrous cyclicity must be applied as early as possible, considering the time necessary to accommodate uterine involution and replenishment of LH stores in the anterior pituitary gland.

A short luteal phase usually occurs after first postpartum ovulation, and treatment with progestogens before first postpartum ovulation consistently reduced the occurrence of short estrous cycles (Sá Filho et al., 2009). Thus, combining pretreatment with progestogens and temporary weaning may enhance the proportion of cows that exhibit estrous behavior and conceive at the first postpartum estrus (Vasconcelos et al., 2009a). Considering the extended length of gestation of *Bos indicus* cattle, treatment with progestogens must be initiated early during the postpartum period and be of short duration, otherwise calving intervals will extend longer than 12 mo.

We designed a series of experiments to establish a melengestrol acetate (MGA)-based estrous synchronization protocol to be used in conjunction with natural service in *Bos indicus* cattle. Our objective was to develop a short-term treatment for *Bos indicus* cattle that induces estrous cyclicity before postpartum loss of body condition, prevents premature luteolysis, eliminates the need for labor required to detect estrus, and consequently increases the likelihood of pregnancy early during the postpartum period.

MATERIALS AND METHODS

All experiments described below were conducted in commercial ranches in Brazil under authority of their managers. Animals were cared for in accordance with the practices outlined in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 1999).

Exp. 1

The objective of this experiment was to determine whether length (7 vs. 13 d) of treatment with MGA...
The objective of this experiment was to determine presence of a CL, and then randomly assigned to receive one of the following treatments: 1) MGA (0.5 mg·cow\(^{-1}·d^{-1}\) of MGA on mineral between d −14 and −1; n = 94); and 2) MGA+ECP (0.5 mg·cow\(^{-1}·d^{-1}\) of MGA on mineral between d −14 and −1, and 2.0 mg intramuscular injection of ECP on d −9; n = 93). On d 0, ultrasound exams were performed to measure the diameter of the largest follicle. Data were analyzed by PROC GLM of SAS, and the model included the effects of treatment within presence of CL on d −14.

**Exp. 3**

The objective of this experiment was to evaluate 2 dosages (1.0 vs. 2.0 mg) of ECP in an estrous synchronization protocol with MGA on estrous detection, conception, and pregnancy rates in multiparous Nelore cows. Postpartum multiparous Nelore cows (n = 399; 60 to 120 d postpartum), with BCS 3.6 ± 1.1 evaluated on d 0 using a BCS scale from 1 = emaciated to 5 = obese (Houghton et al., 1990), were placed on Brachiaria brizantha pasture with water and mineral ad libitum and randomly assigned to receive one of the following treatments: 1) control- no treatment (n = 127); 2) ECP1 (n = 137), 0.5 mg·cow\(^{-1}·d^{-1}\) of MGA on mineral between d −14 and −1, and 1.0 mg i.m. injection of ECP on d −9; 3) ECP2 (n = 135), 0.5 mg·cow\(^{-1}·d^{-1}\) of MGA on mineral between d −14 and −1, and 2.0 mg i.m. injection of ECP on d −9. A 48-h calf removal was performed among cows assigned to the ECP1 and ECP2 treatments, between d 0 and 2. Cows from the 3 treatments were observed for estrous behavior twice per day (morning and evening) between d 0 and 30, and AI were performed 12 h after detected estrus. Pregnancy diagnosis was performed on d 60 by ultrasound examination. Estrous detection, conception, and pregnancy rates were analyzed by PROC LOGISTIC of SAS. Models included the effects of treatment, BCS (covariate), treatment × BCS, AI technician, and AI sire. When treatment effects were detected, Bonferroni’s test was used for mean separation.

**Exp. 4**

The objective of this experiment was to evaluate estrous detection, conception, and pregnancy rates in cycling Nelore heifers treated with MGA and ECP. Nulliparous Nelore heifers (24 to 30 mo old) were placed on Brachiaria humidicola pasture with water and mineral ad libitum and examined by ultrasound to evaluate CL presence at d −15. Heifers with luteal tissue (n = 501) were randomly assigned to receive one of the following treatments: 1) control- no treatment (n = 180); 2) MGA+ECP (n = 321), 0.5 mg·cow\(^{-1}·d^{-1}\) of MGA on mineral between d −14 and −1, and 2.0 mg intramus-
cular injection of ECP on d −9. Between d 0 and 30, heifers were observed for estrus (morning and evening) and inseminated 12 h after estrus detection. Pregnancy diagnosis was performed on d 60 by ultrasonography. Estrus detection, conception, and pregnancy data were analyzed by PROC LOGISTIC of SAS. Models included the effects of treatment, AI technician, and AI sire.

### Exp. 5

The objective of this experiment was to evaluate the effect of treatment with MGA with or without ECP on pregnancy rates (PR) at 10 (PR10), 40 (PR40), and 70 (PR70) d of a natural service breeding season in postpartum Nelore cows. On d −14, 1,029 postpartum Nelore cows were evaluated by ultrasound and blocked according to presence of a CL. Within each block, cows were assigned to receive one of the following treatments: 1) control-no treatment (n = 67 with CL; n = 245 without CL); 2) MGA (0.5 mg·cow−1·d−1 of MGA on mineral between d −10 and −3, followed by treatment similar to control; n = 92 with CL; n = 277 without CL); or 3) MGA+ECP (0.5 mg·cow−1·d−1 of MGA on mineral between d −16 and −3, followed by treatment similar to control; n = 86 with CL; n = 262 without CL). Body condition score was evaluated as described in Exp. 3. Between d 0 and 2 (48 h), calf removal was performed on cows in the MGA and MGA+ECP treatments, and cows from the 3 treatments were exposed to sires for natural service (1 sire per 15 cows) for 70 d. Ultrasound exams for pregnancy diagnosis were performed on d 40, 70, and 100 to evaluate PR10, PR40, and PR70, respectively. Data were analyzed by PROC LOGISTIC of SAS. The model included the effects of treatment, BCS (covariate), and the respective interaction. When treatment effects were detected, Bonferroni’s test was used for mean separation.

### RESULTS

#### Exp. 1

The diameter of the largest follicle at d 0 differed (Table 1; P < 0.01) among treatments. Cows assigned to the MGA13 treatment had the largest follicles; follicle size among MGA7 treated cows was intermediate; control cows had the smallest follicles (11.9, 10.6, and 9.8 mm, respectively). There were also differences among treatments when considering the incidence of premature luteolysis (P < 0.01; Table 1) or short cycles. Pregnancy rates at 10 (PR10), 40 (PR40), and 70 (PR70) d were greater among cows assigned to each of the 2 ECP treatments compared with control cows. Although conception rate was not affected by treatment (P > 0.1), pregnancy rates were increased in cows receiving ECP.

#### Exp. 2

Treatment with ECP (2.0 mg) at d −9 reduced mean follicle diameter at the end of treatment with MGA in cows with (11.10 ± 0.72 vs. 8.08 ± 0.56 mm for MGA and MGA+ECP treated cows, respectively; P < 0.05) or without CL (9.56 ± 0.34 vs. 7.61 ± 0.38 mm for MGA and MGA+ECP treated cows, respectively; P < 0.05).

#### Exp. 3

There was an effect of treatment on estrus detection and pregnancy rate during the first 10 d of the breeding season (P < 0.01; Figure 1; Table 2). Estrus detection rate and pregnancy rate after 10 d was greater among cows assigned to each of the 2 ECP treatments compared with control cows. Although conception rate was not affected by treatment (P > 0.1), pregnancy rates were increased in cows receiving ECP.
during the first 30 d of the breeding season tended to be greater among cows treated with 2.0 mg of ECP ($P < 0.1$; Table 2).

**Exp. 4**

Among heifers assigned to the MGA+ECP treatment (Table 3), 50 of the 115 heifers not detected in estrus during the first 10 d (43.5%) were observed in estrus between d 21 and 28 of the breeding season. Because all heifers were estrous cycling when treatments were initiated, those heifers were not detected in estrus during the first 10 d of the breeding season, but likely ovulated within this period and subsequently returned to estrus synchronously. This indicates that the percentage of heifers ovulating within 10 d after treatment with MGA+ECP was likely greater than 75%.

Similar to results from Exp. 3, conception rates did not differ ($P > 0.1$) between control and MGA+ECP treated heifers. Pregnancy rate during the first 10 d of the breeding season was greater ($P < 0.01$) among MGA+ECP treated heifers because of greater estrous detection rates compared with controls ($P < 0.01$; Table 3; Figure 2). This beneficial effect of treatment with MGA+ECP on pregnancy rate was also observed after the first 30 d of the breeding season ($P < 0.01$; Table 3).

### Table 2. Estrous detection, conception, and pregnancy rates in the first 10 d, and pregnancy rate in 30 d of breeding season in multiparous postpartum Nelore cows (Exp. 3)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Control, %</th>
<th>ECP1, %</th>
<th>ECP2, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrous detection rate (10 d)$^1$</td>
<td>14.9 (19/127)$^a$</td>
<td>48.2 (66/137)$^b$</td>
<td>47.4 (64/135)$^b$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Conception rate (10 d)$^2$</td>
<td>52.6 (10/19)</td>
<td>43.9 (29/66)</td>
<td>45.3 (29/64)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Pregnancy rate (10 d)$^3$</td>
<td>7.9 (10/127)$^a$</td>
<td>21.2 (29/137)$^b$</td>
<td>21.5 (29/135)$^b$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pregnancy rate (30 d)$^4$</td>
<td>30.7 (39/127)$^a$</td>
<td>41.4 (43/137)$^b$</td>
<td>42.2 (57/135)$^b$</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

$^a,b$Means with different letters within a row differ [estrous detection rate: $P < 0.0001$; pregnancy rate (10 d): $P = 0.0037$].

$^1$Means with different letters within a row tend to differ ($P = 0.0891$).

$^1$Cows received no treatment before the beginning of the breeding season.

$^2$Cows received 0.5 mg·d$^{-1}$ of melengestrol acetate (MGA, Pfizer Animal Health, São Paulo, Brazil) on mineral between d $-14$ and $-1$, 1.0 mg intramuscular of estradiol cypionate (ECP, Pfizer Animal Health) on d $-9$, and a 48-h temporary weaning between d 0 and 2 of the breeding season.

$^3$Cows received 0.5 mg·d$^{-1}$ of MGA on mineral between d $-14$ and $-1$, 2.0 mg intramuscular of estradiol cypionate on d $-9$, and a 48-h temporary weaning between d 0 and 2 of the breeding season.

$^4$Percentage of cows detected in estrus in the first 10 d of the breeding season compared with all cows treated.

$^5$Percentage of cows pregnant compared with all cows inseminated during the first 10 d of the breeding season.

$^6$Percentage of cows pregnant after the first 10 d of the breeding season compared with all cows treated.

$^7$Percentage of cows pregnant after 30 d of the breeding season compared with all cows treated.
Exp. 5

Among cows without CL at treatment initiation, pregnancy rates by d 10 and 40 of the breeding season were greater among MGA and MGA+ECP treated groups than for controls ($P < 0.01$; Table 4). However, there was no difference in pregnancy rate between cows assigned to the MGA and MGA+ECP treatments ($P > 0.1$). Pregnancy rate after d 70 of the breeding season did not differ among treatments for cows devoid of CL before treatment initiation ($P > 0.1$).

Among cows with CL, pregnancy rate after d 10 was greater among MGA+ECP treated cows than for cows assigned to the MGA and control treatments ($P < 0.05$; Table 4). Pregnancy rates after d 40 and 70 of the breeding season did not differ among treatments ($P > 0.1$). Regardless of treatment, there was an effect of BCS on pregnancy rates after d 10, 40, and 70 of the breeding season ($P < 0.01$; Figure 3).

**DISCUSSION**

This series of experiments was designed to develop an estrous synchronization protocol to be used in *Bos indicus* cattle under natural service systems. The expectations in determining success of the protocol were based on the following criteria: 1) capability of the protocol to successfully induce estrous cyclicity among anestrous postpartum cows; 2) prevention of premature luteolysis.

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**Table 3. Estrous detection, conception, and pregnancy rates in the first 10 d, and pregnancy rate in 30 d of breeding season in cycling Nelore heifers (Exp. 4)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control,$^1$ %</th>
<th>MGA+ECP,$^2$ %</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrus detection rate (10 d)$^3$</td>
<td>30.5 (55/180)</td>
<td>64.2 (206/321)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Conception rate (10 d)$^1$</td>
<td>50.9 (28/55)</td>
<td>45.6 (94/206)</td>
<td>$&gt;0.1$</td>
</tr>
<tr>
<td>Pregnancy rate (10 d)$^5$</td>
<td>15.5 (25/180)</td>
<td>29.3 (94/321)</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Pregnancy rate (30 d)$^6$</td>
<td>35.5 (64/180)</td>
<td>55.5 (178/321)</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

$^1$Cycling heifers received no treatment before the beginning of the breeding season.

$^2$Cycling heifers received 0.5 mg·d$^{-1}$ of melengestrol acetate (MGA, Pfizer Animal Health, São Paulo, Brazil) on mineral between d $-14$ and $-1$, and 2.0 mg intramuscular of estradiol cypionate (ECP, Pfizer Animal Health) on d $-9$ of the breeding season.

$^3$Percentage of heifers detected in estrus during the first 10 d of the breeding season compared with all heifers treated.

$^4$Percentage of heifers pregnant compared with all heifers inseminated during the first 10 d of the breeding season.

$^5$Percentage of heifers pregnant in the first 10 d of the breeding season compared with all heifers treated.

$^6$Percentage of heifers pregnant in 30 d of the breeding season compared with all heifers treated.
after the first postpartum ovulation; 3) conception rate equivalent to a spontaneous estrus with no reduction in pregnancy occurring as a result of treatment; and 4) improvement in pregnancy rates early in the breeding season. Melengestrol acetate-based protocols are useful tools to be used in conjunction with natural service mating systems for beef cows because oral administration of the progestogen is practical and requires limited management of the animals. Most MGA-based protocols consist of long-term treatment (18 to 31 d), which in many cases limits their use in herds of postpartum beef cows. Because the length of postpartum anestrous in *Bos indicus* beef cows is typically extended and BCS decreases as days postpartum increase, a short-term protocol that induces estrous cyclicity and fulfills the requirements previously stated is necessary.

Patterson et al. (2003a,b) proposed the general hypothesis that MGA treatment before a GnRH-PGF$_2\alpha$ estrous synchronization protocol would successfully 1) induce ovulation in anestrous postpartum beef cows; in many cases limits their use in herds of postpartum beef cows. Because the length of postpartum anestrous in *Bos indicus* beef cows is typically extended and BCS decreases as days postpartum increase, a short-term protocol that induces estrous cyclicity and fulfills the requirements previously stated is necessary.

Patterson et al. (2003a,b) proposed the general hypothesis that MGA treatment before a GnRH-PGF$_2\alpha$ estrous synchronization protocol would successfully 1) induce ovulation in anestrous postpartum beef cows;

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### Table 4. Pregnancy rates at 10, 40, and 70 d of breeding season in postpartum Nelore cows according to the presence of corpus luteum (CL) at beginning of treatments (Exp. 5)

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Control, 1 %</th>
<th>MGA, 2 %</th>
<th>MGA+ECP, 3 %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows without CL$^4$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate$^5$ (10 d)</td>
<td>Control, 1 %</td>
<td>11.0 (27/245)$^a$</td>
<td>36.1 (100/277)$^b$</td>
<td>38.2 (100/262)$^b$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pregnancy rate (40 d)</td>
<td>MGA, 2 %</td>
<td>36.7 (90/245)$^a$</td>
<td>61.4 (170/277)$^b$</td>
<td>56.9 (149/262)$^b$</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pregnancy rate (70 d)</td>
<td>MGA+ECP, 3 %</td>
<td>64.5 (158/245)</td>
<td>69.7 (193/277)</td>
<td>67.6 (177/262)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Cows with CL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate (10 d)</td>
<td>Control, 1 %</td>
<td>38.8 (26/67)$^a$</td>
<td>33.7 (31/92)$^a$</td>
<td>53.5 (46/86)$^b$</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pregnancy rate (40 d)</td>
<td>MGA, 2 %</td>
<td>64.2 (43/67)</td>
<td>65.2 (60/92)</td>
<td>73.2 (63/86)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Pregnancy rate (70 d)</td>
<td>MGA+ECP, 3 %</td>
<td>85.1 (57/67)</td>
<td>72.8 (67/92)</td>
<td>84.8 (73/86)</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

$^a,b$Means with different letters within a row differ (cows without CL: $P < 0.0001$; cows with CL: $P = 0.0226$).

$^1$Cows received no treatment before the beginning of the breeding season.

$^2$Cows received 0.5 mg·d$^{-1}$ of melengestrol acetate (MGA, Pfizer Animal Health, São Paulo, Brazil) on mineral between d $-14$ and $-1$, and a 48-h temporary weaning between d 0 and 2 of breeding season.

$^3$Cows received 0.5 mg·d$^{-1}$ of MGA on mineral between d $-14$ and $-1$, 2.0 mg intramuscular of estradiol cypionate (ECP, Pfizer Animal Health) on d $-9$, and a 48-h temporary weaning between d 0 and 2 of the breeding season.

$^4$Cows with absence of luteal tissue assessed 14 d before beginning of the breeding season.

$^5$Percentage of cows pregnant in the first 10, 40, or 70 d of the breeding season compared with all cows treated.
2) reduce the incidence of a short luteal phase among anestrous cows induced to ovulate; 3) increase estrous response, synchronized conception, and pregnancy rate; and 4) increase the likelihood of successful fixed-time AI. Protocols that utilize this sequential approach for control of the estrous cycle in cows include the MGA Select (Perry et al., 2002b; Stegner et al., 2004) and 7–11 Synch (Kojima et al., 2000) protocols. Although these protocols were used successfully in postpartum beef cows in breeding programs involving AI, there are drawbacks to their use in facilitating synchronization of estrus before natural service.

The simplest reported method for using MGA to synchronize estrous cycles in cattle involves using bulls to breed synchronized groups of females (Patterson et al., 2002). This practice was useful in helping producers make a transition from natural service to AI. In this process, cows or heifers received the normal 14-d feeding program of MGA and were then exposed to fertile bulls about 10 d after MGA withdrawal. The primary reason for the extended length of this protocol pertains to the reduction in fertility among cows that exhibit estrus after MGA withdrawal, which in most cases is associated with the development of persistent follicles during MGA feeding. Hence, a major disadvantage to this system pertains to the overall length of treatment and confirms the importance of pursuing alternative strategies to successfully utilize MGA in breeding programs involving estrus synchronization and natural service.

In Exp. 1 we evaluated 2 short-term treatments with MGA on premature luteolysis after first postpartum ovulation in Nelore cows. It is well known that a minimum of at least 3 d of progestogen exposure before the first postpartum ovulation is required to ensure normal luteal lifespan (Sá Filho et al., 2009), conception, and pregnancy (Vasconcelos et al., 2009a). Treatment with MGA (0.5 mg·cow⁻¹·d⁻¹) for 7 d prevented premature luteolysis in only 61.5% of treated cows, whereas treatment for 13 d prevented premature luteolysis in 100% of treated cows. In previous reports, treatments with 0.5 mg·d⁻¹ of MGA for 5 d (Smith et al., 1987) or 7 d (Perry et al., 2004), or treatment with 4.0 mg·d⁻¹ for 7 d (Perry et al., 2004) before first postpartum ovulation prevented premature luteolysis in only 46, 0, and 29% of cows, respectively, indicating that different progestogens might not have the same biological activity, likely due to variations in the affinity of the progestosterone receptor (Perry et al., 2002a).

Diameter of the largest follicle at the time of GnRH treatment was increased as the length of MGA treatment increased in Exp. 1, indicating that MGA treatment maintains follicular growth for a longer period of time in anestrous cows. Persistent ovarian follicles generally form among estrous cycling cows without CL at treatment initiation when progestogens are administered in doses used commercially (Sirois and Fortune, 1990; Cupp et al., 1992; Stock and Fortune, 1993). Persistent follicles form as a result of increased pulse frequency of LH, similar to pulse frequency patterns of LH during the follicular phase of the estrous cycle (Roberson et al. 1989; Kojima et al., 1992; Sanchez et al., 1995).

Interestingly, treatment with MGA induced formation of persistent follicles only among estrous cycling cows, but not among anestrous cows (Perry et al., 2002c). Because cows with persistent follicles ovulate oocytes of poorer quality (Rehav and Butler, 1996; Mihm et al., 1999) and experience alterations in oviductal-uterine environments (Ahmad et al., 1995; Shaham-Albalancy et al., 1997; Binelli et al., 1999; Borchert et al., 1999), fertility among these cows is reduced (Sanchez et al., 1993; Wehrman et al., 1993; Anderson and Day, 1994; Mihm et al., 1994; Ahmad et al., 1995; Kinder et al., 1996). Therefore, strategies to synchronize the development of follicular waves are necessary in using MGA-based protocols especially when considering administration to estrous cycling beef cows.

Several studies are reported that were designed to induce follicular atresia among cows with persistent follicles (Ireland and Roche, 1982; Roberson et al., 1989; Anderson and Day, 1994, 1998; McDowell et al., 1998). Another way to induce follicular atresia among cows with persistent follicles is the combination of progestogens and estradiol. Treatment with progesterone + estradiol was shown to effectively suppress FSH and follicular growth and successfully synchronize follicular wave emergence in the bovine female regardless of stage of the follicular phase (Bo et al., 1994). Although this treatment results in a range (3 to 6 d) in time over which follicular waves begin after treatment (Burke et al., 2000; Martinez et al., 2000; Diskin et al., 2002; Kim et al., 2005), the combined treatment of progesterone and estradiol resulted in satisfactory results among both anestrous and estrous cycling beef cows (Fike et al., 1997; Rhodes et al., 2002). Administration of estradiol valerate during a 14-d treatment with MGA prevented the development of persistent dominant follicles (Yelich et al., 1997). This most likely occurred as a result of the negative effect of estradiol valerate on the hypothalamic-pituitary axis (Engelhardt et al., 1989), the corresponding decreased number of LH pulses, and resulting atresia of the dominant follicle (Rajamahendran and Manikkam, 1994). Similarly, cows that received an intramuscular injection of estradiol-17β + progesterone at the beginning of a 6-d treatment with MGA had smaller dominant follicles than cows that received only MGA (Martinez et al., 2001). Results of Exp. 2, in which ECP on d −9 reduced follicle diameter at the end of MGA treatment among both estrous cycling and anestrous cows, indicates that ECP was effective in preventing the development of persistent follicles. These data furthermore indicate that ECP used in combination with MGA has the potential to prevent reduction in fertility that generally occurs among cows mated at the first estrus immediately after withdrawal of MGA.

In Exp. 3 and 4, we evaluated the effects of MGA treatment on fertility of multiparous Nelore postpartum
cows and estrous cycling heifers followed by AI. The main objective of these experiments was to determine whether treatment with MGA in combination with ECP would negatively impact conception, or whether results would agree with previous studies (Anderson and Day, 1998; McDowell et al., 1998). In Exp. 3, treatment with MGA + ECP improved estrus detection rate during the first 10 d of the breeding season. This effect may be attributed to 1) reduced estradiol negative feedback on LH through site-specific reductions in hypothalamic neurons that contain receptors for estradiol (Anderson and Day, 1996) during MGA treatment; 2) synchronization of follicular waves after ECP treatment, which may concentrate or synchronize the distribution of estrus activity during the first days of the breeding season; or 3) increased estrous response with temporary weaning; or 3) increased estrous response with temporary weaning, which stimulates follicular growth and cows may be due to the fact that cows received differences in patterns of estrus activity among heifers most heifers exhibited estrus between d 3 and 5. These ex- cessive fertile activity during the first days of the breeding season. These data are in agreement with the idea that adequate BCS at calving is critical for satisfactory fertility in suckled beef cows (Meneghetti and Vasconcelos, 2008). Cows experience a period of negative energy balance after calving that makes them more sensitive to the negative feedback effect of estradiol on gonadotropin release (Schillo et al., 1992). Thus, cows with greater BCS after calving are more likely to respond favorably to strategies designed to induce estrous cyclicity (Pinheiro et al., 1998; Wiltbank et al., 2002; Vasconcelos et al., 2009a).

Analysis of estrus distribution plots of suckled cows and estrous cycling heifers indicated that most cows exhibited estrus on d 2 of the breeding season, whereas most heifers exhibited estrus between d 3 and 5. These differences in patterns of estrus activity among heifers and cows may be due to the fact that cows received temporary weaning, which stimulates follicular growth (Vasconcelos et al., 2009b) and subsequently affected the timing of estrus expression. Furthermore, the possibility of a reduced rate of MGA clearance among heifers may not be ruled out.

In conclusion, we obtained satisfactory pregnancy rates (~40%) in Bos indicus cattle with the following protocol: 0.5 mg·d^-1 of MGA between d −14 and −1; 2.0 mg intramuscular injection of ECP on d −9; 48 h temporary weaning between d 0 and 2; natural service beginning on d 0 (summarized in Figure 4). This protocol effectively induced estrous cyclicity among anestrous cows, synchronized estrus activity, and prevented premature luteolysis with no negative effect on conception. Factors potentially affecting pregnancy rates among cows submitted to this treatment (e.g., semen quality of sires, bull to female ratios, BCS, and parity of cows) should be considered to anticipate and improve results.
LITERATURE CITED


