Impact of puberty status and melengestrol acetate supplementation before the breeding period on reproductive efficiency of *Bos indicus* beef heifers[1]

J. H. Martins,* C. S. Santos,* M. A. V. Silva,* H. M. V. S. B. Aguiar,† I. G. França,† H. G. Pereira,† D. L. Ribeiro,* R. M. Chaves,* J. A. T. Souza,‡ B. M. Monteiro,§ M. F. Sá Filho,§ and J. R. S. Torres-Júnior*†[2]

*Agrarian Sciences Center, State University of Maranhão, CCA-UEMA, São Luís-MA, Brazil, 65055310; †Agrarian and Environmental Sciences Center, Federal University of Maranhão, CCAA-UFMA, Chapadinha-MA, Brazil, 65500000; ‡Agrarian Sciences Center, Federal University of Piauí, CCA-UFPI, Teresina-PI, Brazil, 64049550; and §Department of Animal Reproduction (VRA), FMVZ-USP, São Paulo, Brazil, 05508270

ABSTRACT: Two experiments were designed to evaluate the impact of puberty status and the administration of melengestrol acetate (MGA) before onset of the breeding period on ovulatory responses (Exp. 1) and conception rate after AI performed on estrus detection during 10 d and the pregnancy rate through 80 d of breeding period (Exp. 2) of pasture-grazed *Bos indicus* beef heifers. In Exp. 1, heifers (15 pubertal and 15 prepubertal) received 0.5 mg per heifer/d^{-1} –1 of MGA over 14 d. No differences in the ovulatory responses were found 10 d after the MGA administration (pubertal = 46.7% vs. prepubertal = 53.3%; P = 0.72). In Exp. 2, 368 heifers were randomly assigned to groups according to pubertal status and the MGA treatment. All heifers were inseminated on estrus detection for up 10 d after MGA administration and following exposure to bulls between 20 and 80 d. The MGA-treated heifers exhibited a greater AI service rate than control heifers (72.1 vs. 41.6%; P < 0.01); however, heifers receiving MGA had lower conception results following AI (51.6 vs. 71.4%; P = 0.01). In addition, MGA-treated heifers were more likely to have a corpus luteum in the middle of the breeding period (95.3 vs. 87.5%; P < 0.01), although the Cox proportional hazard of pregnancy rate was similar (P = 0.29) at the end of the breeding period. At onset of the breeding period, pubertal heifers presented a greater pregnancy rate following AI (pubertal = 42.2% vs. prepubertal = 24.9%; P = 0.01). Therefore, pubertal heifers seem to have greater overall reproductive efficiency than prepubertal heifers, particularly at the beginning of the breeding period. Interestingly, administration of MGA before the onset of the breeding period increased AI service rate but did not alter the rate of pregnancy throughout the breeding period of pasture-grazed *Bos indicus* beef heifers.

Key words: artificial insemination, cyclicity, follicular dynamics, heifers, progestin

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INTRODUCTION

The age at puberty in heifers represents an important factor to determine whether animals will remain in the herd and their lifetime productivity (Cushman et al., 2013; Ferrell, 1982; Funston et al., 2012; Patterson et al., 1992). Therefore, strategies to shorten age at puberty to increase the proportion of cycling heifers at the onset of the breeding period could be particularly important to *Bos indicus* heifers, known to reach puberty at older ages (22 to 36 mo; Nogueira, 2004).

Melengestrol acetate (MGA) is a commercially available progestin that blocks the preovulatory surge

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[2]Corresponding author: jrtorresjr@gmail.com
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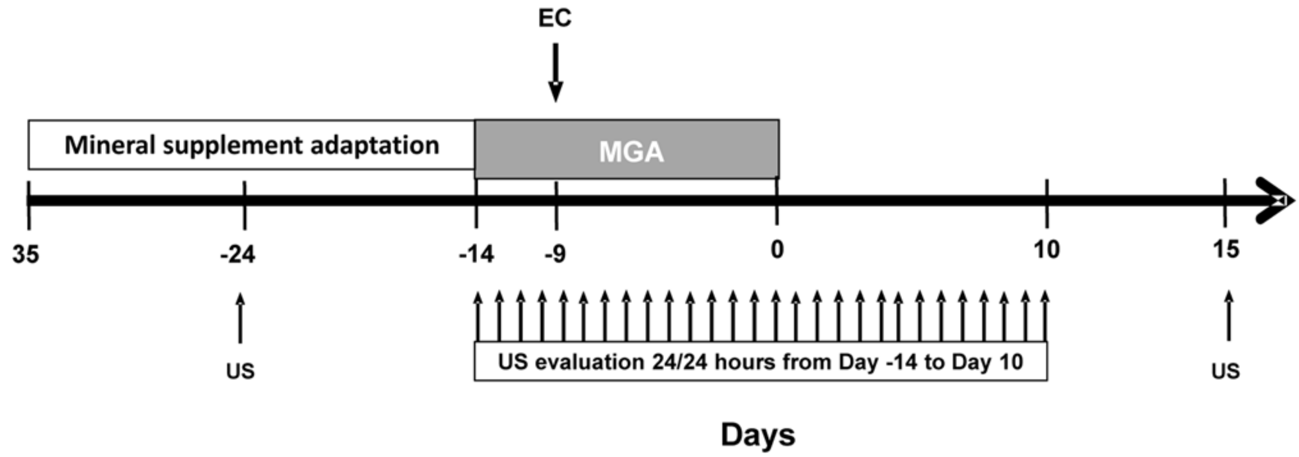

Figure 1. Treatment schedule of Exp. 1. EC = estradiol cypionate; MGA = melengestrol acetate; US = ultrasound examination.

of LH and the ovulation of heifers during the supplementation period (Patterson et al., 1989; Imwalle et al., 2002; Wood-Follis et al., 2004; Perry et al., 2005; Tauck et al., 2007).

A recent study, using *Bos indicus* cattle kept in pastures, showed a positive effect of MGA treatment on reproductive efficiency of both suckled cows and cyclic heifers (Sá Filho et al., 2009). However, this elegant study did not demonstrate the impact of MGA supplementation on prepubertal *Bos indicus* heifers. Therefore, the efficacy of this protocol according to the puberty status of *Bos indicus* heifers has not been thoroughly evaluated.

Furthermore, despite the vast literature regarding the use of MGA supplementation for estrous synchronization of *Bos taurus* (Patterson et al., 1989), *Bos indicus* (Sá Filho et al., 2009), and *Bos indicus*–crossed beef heifers (Bridges et al., 2005; McKinniss et al., 2011), relatively little information is available concerning the impact of MGA supplementation before breeding on induction of puberty and the rate of pregnancy throughout the breeding period in pasture-grazed *Bos indicus* heifers.

Therefore, the aim of the present study was to evaluate the effects of MGA administered before the breeding period on ovarian follicular dynamics (Exp. 1) and on estrus synchronization and pregnancy rate throughout the breeding period (Exp. 2) of *Bos indicus* beef heifers.

MATERIALS AND METHODS

Experiment 1: Effects of Melengestrol Acetate on the Ovarian Follicular Dynamics

Location and Animals. This experiment was performed in a commercial beef farm (8°28′41″ S, 46°36′54″ W) located in Balsas, Maranhao, Brazil. All heifers were kept on a *Brachiaria brizantha* pasture and given mineral salt and free access to water. A total of 30 Nelore (*Bos indicus*) beef heifers between 20 and 24 mo of age, weighing 295 ± 1.4 kg, and having a BCS (Ayres et al., 2009) of 3.1 ± 0.3 were enrolled in the study. Heifers at that age were selected because puberty usually occurs between 22 and 36 mo of age in this cattle breed (Nogueira, 2004).). The experimental protocol was reviewed and approved by the Ethics Committee on the use of animals from the Federal University of Maranhao, Brazil (protocol no. 23115008344/2011-21).

Mineral and Melengestrol Acetate Supplementations. Twenty-one days before the onset of the trial (Day –35 to –14; Fig. 1), all heifers were subjected to a period of supplement adaptation and intake adjustment. A mineral mixture recommended for cattle before the breeding period (Prebreeding Premix; Pfizer, Sao Paulo, Brazil) was offered ad libitum to all heifers. During the last 7 d of this adaptation period, the average daily ad libitum intake of the mineral supplement for animals was evaluated. If necessary, the amount of supplement offered per day was adjusted so that most of the bunk floor was visible within 24 h after supplement delivery. This methodology was based on typical feedlot bunk management as previously described by Pritchard (1998).

Based on the daily intakes of the mineral mixture over the 7 d before the onset of the trial (120 g per heifer/d), 0.5 mg of MGAper heifer/d (2.28 mg of MGA Premix; Zoetis Animal Health, Sao Paulo, Brazil) was included in the mineral supplement of the MGA treatment group, regardless of pubertal status.

The MGA supplementation was provided during 14 d (Day –14 to 0; Fig. 1). Then, on the fifth day of MGA supplementation (Day –9), all heifers were treated with 1 mg of estradiol cypionate (**EC**) intramuscular

(ECP; Zoetis Animal Health) to induce follicular atresia and emergence of a new follicular wave.

Experimental Design and Ultrasonography Examination. All heifers were classified according to pubertal status (prepubertal [$n = 15$] and pubertal [$n = 15$]) following 2 transrectal ultrasonography examinations (5 MHz; Chison 500VET; Kylumax Eletromedicina LTDA, Sao Paulo, Brazil). The ultrasound exams were performed on Day –24 and –14 of the trial period (Fig. 1). Heifers that had a corpus luteum (**CL**) in at least 1 of the ultrasound exams were classified as pubertal. On Day –24, heifers had their BW and BCS recorded.

To evaluate the ovarian follicular dynamics during the MGA treatment, heifers were subjected to ovarian ultrasound evaluations every 24 h from the beginning of the MGA supplementation (Day –14) until 10 d after the end of the progestin supplementation (Day 10). Follicular growth rate (mm/d) of the dominant follicle (**DF**) was also recorded starting at the end of MGA supplementation (Day 0) until ovulation. An additional ultrasound examination was performed to verify the formation of a CL following the progestin treatment (Day 15).

Experiment 2: Effects of Melengestrol Acetate on the Estrus Synchronization and Pregnancy Rate

Location and Animals. This experiment was conducted in a commercial beef farm (5°40′00″ S, 43°32′06″ W) located in Parnarama, Maranhao, Brazil. All heifers were kept on a *Brachiaria brizantha* pasture and given mineralized salt and free access to water. A total of 368 Nelore (*Bos indicus*) beef heifers between 20 and 24 mo of age with average BW of 295 ± 1.4 kg (corresponding to approximately 65% of mature BW) and BCS of 3.1 ± 0.3 were enrolled in the study.

Heifers had their BW recorded at onset of the breeding period. Body weights were obtained using a digital scale and animals were without any period of food or water restriction. To analyze the relationship between BW and reproductive responses, animals were classified according to their BW on the first day of the breeding period as light (BW < 275 kg; $n = 95$), moderate (BW between 275 and 300 kg; $n = 110$), or heavy (BW > 300 kg; $n = 163$), based on the mean weight of puberty for *Bos indicus* heifers, as reviewed by (Burns et al., 2010).

Experimental Design and Ultrasound Exams. All heifers were classified according to pubertal status (prepubertal [$n = 190$] and pubertal [$n = 178$]) following 2 transrectal ultrasonographic exams performed at an interval of 10 d (Day –24 and –14; Fig. 2). Only heifers that had a CL in at least 1 of the 2 ultrasound examinations were classified as pubertal. On Day –14, prepubertal and pubertal heifers were randomly assigned to 1 of the 2 groups according to MGA supplementation (MGA; $n = 183$ and no MGA; $n = 185$). Puberty status was used as a block in the experimental design and prepubertal heifers received MGA ($n = 97$) or not ($n = 93$). Similarly, the pubertal heifers received MGA ($n = 86$) or not ($n = 92$). The MGA supplementation was performed as described during Exp. 1. Heifers consumed 120 g·heifer^{-1}·d^{-1} of mineral supplement containing MGA corresponding to an intake of 0.5 mg·heifer^{-1}·d^{-1} of MGA. All heifers were kept in the same pasture throughout the experiment, with the exception of the period of supplementation with MGA, when the MGA-supplemented group was kept in a pasture just beside the no-MGA group.

The onset of the breeding period was defined as the last day of MGA treatment (Day 0). From Day 0 to 10 of the breeding period, on estrus detection, heifers were subjected to AI performed twice daily, in the morning and the evening. At Day 20 of the breeding period, 19 healthy bulls between 3 and 5 yr of age were placed together with the heifers at a proportion of 1 bull for every 20 heifers (1:20). Bulls were kept with heifers until Day 80 of the breeding period. Only bulls classified as potential satisfactory breeders were used, according to guidelines of the Brazilian College of Animal Reproduction (CBRA, 1998).

Three additional ultrasound examinations were performed (Fig. 2). The first exam was performed 30 d after the end of the AI period (Day 40), the second was performed at the end the breeding period (Day 80), and the third was performed 30 d afterward (Day 110). These exams aimed to verify 1) conception rate following the AI, 2) the pregnancy rate after the AI period, 3) the cyclicity at Day 40 of the breeding period, and 4) pregnancy rate at the end of breeding period.

Statistical Analyses

In both Exp. 1 and 2, all statistical analyses were performed using the GLIMMIX procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC) for Windows. Continuous variables were analyzed by ANOVA using the GLIMMIX procedure fitted to normal distribution. Categorical data were analyzed by logistic regression with models fitted to binary distribution.

In Exp. 1, the explanatory variable that was included in the statistical model was pubertal status (prepubertal vs. pubertal). The dependent variables, which included the diameter of the largest follicle at the end of MGA (mm), the maximum diameter of the DF after the removal of MGA (mm), and the growth rate of the DF (mm/d), were tested for the homogeneities and normality of their variances using the Guide Data Analysis from SAS and transformed when necessary. Bartlett's test was used to verify the dispersion of

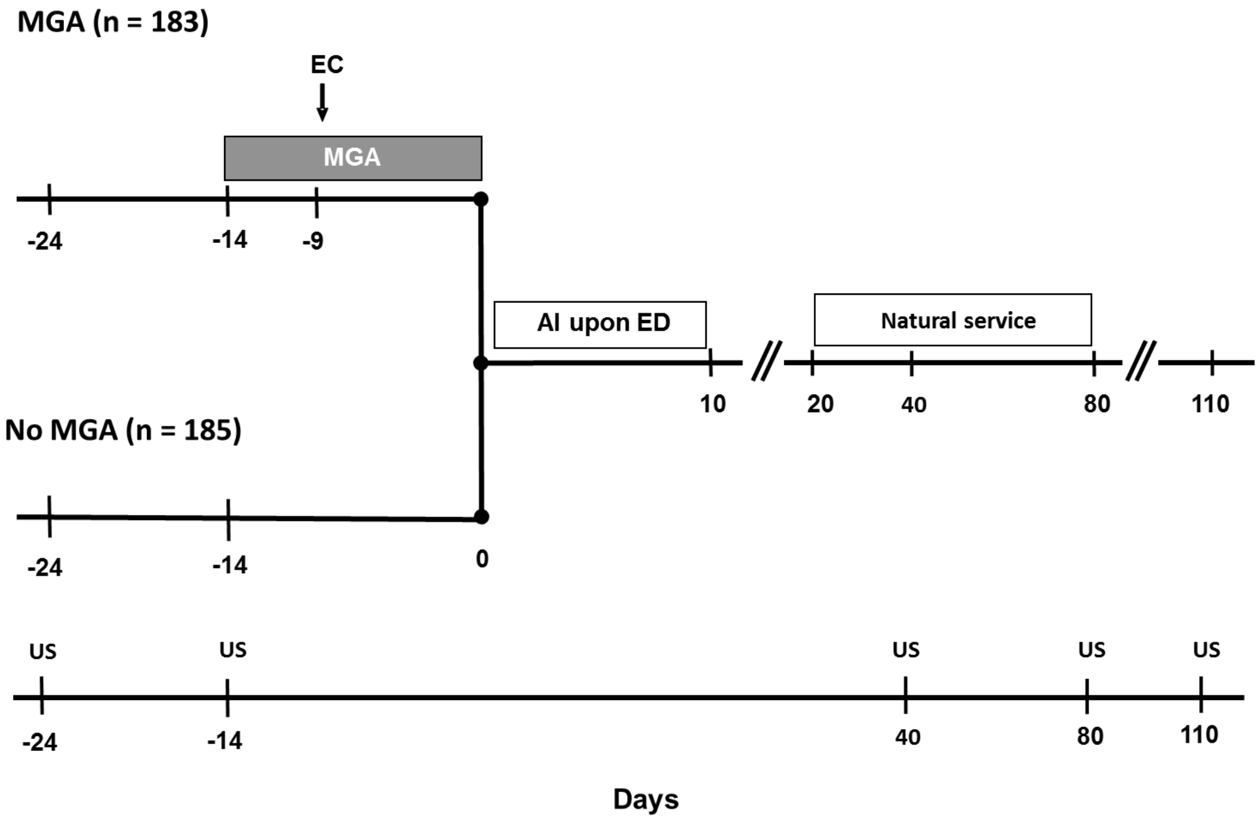

Figure 2. Treatment schedule of Exp. 2. EC = estradiol cypionate; MGA = melengestrol acetate; ED = estrus detection; US = ultrasound examination.

the ovulations. Binomial distributions were assumed for the categorical response variables. The ovulation rate after MGA treatment was also analyzed by the presence of a CL on Day 15.

In Exp. 2, variables included in the models were treatment with MGA, pubertal status (i.e., the presence of a CL before the breeding period), weight class, and meaningful interactions between these 3 variables. The BCS at the beginning of breeding period was included in the model as linear effect. For the final model, variables were removed through backward elimination when $P > 0.20$ based on the Wald's statistical criterion. The dependent variables analyzed in this experiment were the interval between MGA removal and estrus (d), AI service rate during the first 10 d of the breeding period (%), conception rate (number of heifers pregnant by number of heifers inseminated; %), pregnancy rate following AI (number of heifers pregnant by total number of heifers enrolled in the trial; %), and pregnancy rate at the end of breeding period (number of heifers pregnant at the end of breeding period by total of heifers enrolled in the trial; %). The variable cyclicity at Day 40 of the breeding period (%) was analyzed using a model that disregarded pubertal status and weight classes.

Furthermore, the rate of pregnancy was analyzed with the Cox proportional hazard model using the PHREG procedure of SAS. The full model included the fixed effects of treatment (no MGA or MGA), pubertal status (prepubertal or pubertal), BW class (light, moderate, and heavy), and the interactions between treatment and the other covariates. The time variable was the interval in days from the start of the breeding period (Day 0) to pregnancy. For this analysis, heifers that were not pregnant at the end of breeding period were censored. The rate of pregnancy (adjusted hazard ratio) estimated the relative rate of pregnancy according to the explanatory variables used. Median and mean days to pregnancy were obtained from the LIFETEST procedure of SAS. Survival plots were generated with MedCalc version 9.2 (MedCalc Software, Mariakerke, Belgium).

Least squares means ± SE are used to describe the response variables. The $P \leq 0.05$ was considered to be statistically significant and $0.05 < P < 0.10$ was considered to indicate a trend toward difference for the variables evaluated.

RESULTS

Experiment 1: Effects of Melengestrol Acetate on the Ovarian Follicular Dynamics

Daily consumption of MGA that was added to the mineral mixture was considered satisfactory, because there were no significant amounts of leftovers throughout

Table 1. Ovarian follicular responses following melengestrol acetate (MGA) supplementation according to the pubertal statuses of the *Bos indicus* beef heifers (Exp. 1)

Parameter[1]	MGA Prepubertal	MGA Pubertal	P-value
No. of heifers	15	15	–
Time of emergence of DF relative the beginning of MGA supplementation, d	10.2 ± 5.0	8.6 ± 5.0	0.03
Growth rate of the DF from the emergence to the end of MGA supplementation, mm/d	0.5 ± 0.1	0.4 ± 0.1	0.30
Growth rate of the DF from the end of MGA supplementation to ovulation, mm/d	0.4 ± 0.1	0.5 ± 0.1	0.50
Growth rate of the DF from the emergence to ovulation, mm/d	0.5 ± 0.1	0.5 ± 0.1	0.85
Diameter of the LF at the end of MGA, mm	5.5 ± 0.5	5.2 ± 0.4	0.73
Maximum diameter of the DF after MGA removal, mm	8.4 ± 0.4	7.9 ± 0.5	0.52
Ovulation rate, %	53.3%	46.7%	0.72
Time of ovulation relative the end of MGA treatment, d	7.9 ± 0.9	6.4 ± 0.8	0.26
Persistence of the DF, d (interval from follicular emergence to ovulation)	11.6 ± 0.9	10.9 ± 1.2	0.60

[1]DF = dominant follicle; LF = largest follicle.

the study. The results of ovarian follicular responses of this experiment are summarized in Table 1. Prepubertal heifers had a delayed emergence of DF relative to the beginning of MGA supplementation compared with pubertal animals ($P = 0.03$). There were no differences ($P > 0.05$) in persistence of ovulatory follicle (length of ovulatory follicular wave) or in ovulatory responses based on pubertal status at onset of the MGA supplementation. The distributions of ovulation at the end of the MGA supplementation were also similar across pubertal statuses (Fig. 3) in terms of both average time to ovulation ($P = 0.26$) and the dispersion of ovulation ($P = 0.75$).

Experiment 2: Effects of Melengestrol Acetate on the Estrus Synchronization and Pregnancy Rate

Similarly to Exp. 1, daily consumption of MGA added to the mineral mixture was considered satisfactory based on leftovers. There were no interactions between MGA treatment and pubertal status for any of the variables evaluated (Table 2). Heifers treated with MGA experienced estrus earlier than no-MGA heifers (5.9 ± 0.2 vs. 8.0 ± 0.4 d; $P < 0.01$). As a result, the service rate in the first 10 d of the breeding period was greater in MGA-treated heifers than in no-MGA heifers (72.1 vs. 41.6%, respectively; $P < 0.01$). However, lower conception rate (MGA = 51.6% vs. no MGA = 71.4%; $P = 0.01$) was observed in MGA-treated heifers. Still, pregnancy rates were similar between the 2 experimental groups ($P = 0.29$) throughout the breeding period. Despite the greater ($P < 0.01$) proportion of cyclic heifers at Day 40 of the breeding period in the MGA group (95.3%; 162/170) than in the no-MGA group (87.5%; 161/184), similar pregnancy rates were observed at Day 40 (57.4 vs. 52.7%; $P = 0.53$) and 80 (75.4 vs. 73.0%; $P = 0.55$) of the breeding period (Fig. 4).

Pubertal heifers at the beginning of the breeding period exhibited greater general reproductive efficiency than did prepubertal heifers (Table 2). These differences were verified by the greater service rate (67.9 vs. 46.5%; $P < 0.01$) and increased AI conception rate (42.2 vs. 24.9%; $P < 0.01$). Moreover, pubertal heifers exhibited a 30% enhancement in pregnancy rate (84.3 vs. 64.7%; $P = 0.07$) compared with prepubertal heifers. Accordingly, the median number of days to pregnancy was reduced by 10 d for pubertal heifers (31 vs. 41 d; Fig. 5).

When heifers were classified according to their BW at the beginning of the breeding period, the reproductive performance of animals weighing >300 kg was found to be significantly greater (Table 3). Moreover, among the heifers that were heavier at the beginning of the breeding period, a greater proportion were classified as pubertal on Day 40 of the breeding period and the rate of pregnancy of this group was also increased.

DISCUSSION

The present study have shown that regardless of pubertal status (Exp. 1), the ovulatory responses of *Bos*

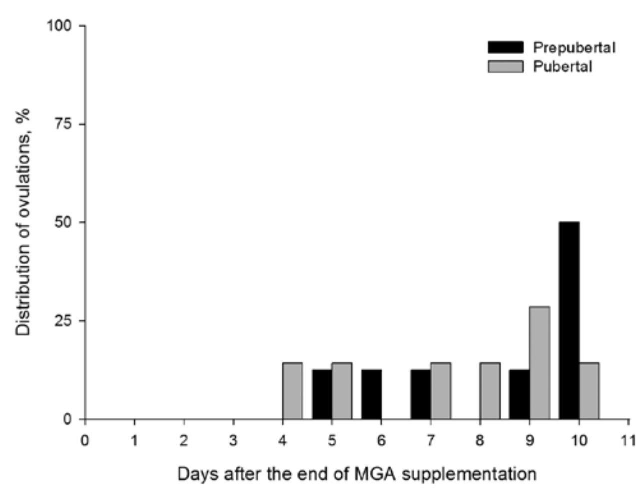

Figure 3. Distribution of ovulations after the end of melengestrol acetate (MGA) supplementation according pubertal status (prepubertal and pubertal). Time of ovulation, $P = 0.26$. Bartlett's test, $P = 0.75$ (Exp. 1).

Table 2. Reproductive parameters according to pubertal status and melengestrol acetate (MGA) supplementation at the beginning of the breeding period of the *Bos indicus* heifers (Exp. 2)

	Treatment[1]						
	MGA		No MGA		*P*-value		
Parameter	Prepubertal	Pubertal	Prepubertal	Pubertal	MGA treatment	Pubertal status	MGA treatment × pubertal status
No. of animals	97	86	93	92	–	–	–
Interval from onset of breeding period to estrus detection,[2] d	5.6 ± 0.3	6.2 ± 0.3	7.9 ± 0.6	8.0 ± 0.5	<0.01	0.78	0.31
Service rate,[3] %	61.9	83.7	31.2	52.2	<0.01	<0.01	0.57
Conception rate,[4] %	50.0	52.9	62.1	77.1	0.01	0.24	0.41
AI pregnancy rate,[5] %	30.3	44.4	19.6	40.2	0.15	0.01	0.44
Pregnancy rate at end of breeding period, %	67.0	84.9	62.4	83.7	0.55	0.07	0.80
Median days to pregnancy	36.0 ± 3.3	26.0 ± 3.1	51.0 ± 3.0	36.0 ± 2.9	0.29	0.01	0.99

[1]The heifers were classified according pubertal status (i.e., the presence of a corpus luteum before the onset of the breeding season). Then, the prepubertal and pubertal heifers were randomly assigned to 1 of 2 groups that received or did not receive 14 d of MGA supplementation in a factorial 2 × 2 design.

[2]Estrus was detected twice daily during first 10 d of the breeding period (i.e., 10 d after the end of the MGA treatment from MGA treatment group).

[3]Service rate = number of inseminated heifers by number of heifers enrolled in the trial.

[4]Conception rate = number of pregnant heifers by number of heifers inseminated during the first 10 d of the breeding period.

[5]AI pregnancy rate = number of heifers that became pregnant following AI by total of heifers enrolled in the trial.

indicus beef heifers after supplementation with MGA were fairly similar. Interestingly, *Bos indicus* heifers that were treated with MGA before the onset of the breeding period exhibited a greater service rate during the first 10 d after the end of the treatment and a greater cyclicity rate at the middle of the breeding period compared with the untreated animals. Despite this improvement, MGA treatment did not alter the rate of pregnancy throughout the breeding period. Additionally, regardless of the MGA treatment, beef heifers that were heavier (>300 kg) and those classified as pubertal at the beginning of the breeding period exhibited greater pregnancy rate throughout the breeding period.

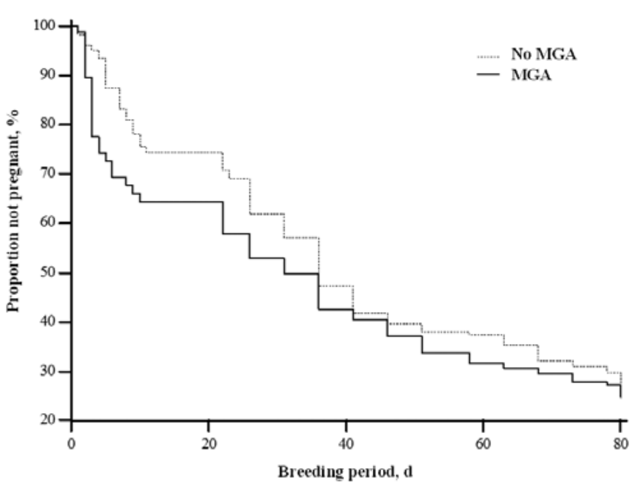

Figure 4. Survival curves for the proportions of nonpregnant heifers by Day 80 of the breeding period for the heifers that were not treated with melengestrol acetate (MGA; No MGA; dashed line; $n = 183$) and those that were treated with MGA (solid line; $n = 185$) before the beginning of the breeding period. The median intervals to pregnancy for the no-MGA and MGA groups were 36 and 31 d (adjusted hazard ratio = 1.14; 95% confidence interval = 0.90 to 1.45), respectively.

The small diameter of the DF at the end of MGA treatment and delayed ovulation in relation to MGA removal that was found in the present study may be due to the effects of the EC administered during the middle of the protocol. Treatment with progesterone/progestagen plus estradiol was shown to effectively suppress LH and FSH and, thereby, follicular growth and to synchronize follicular wave emergence in cattle regardless of stage of the follicular phase (Bó et al., 1994; Haughian et al., 2004). However, LH peaks occurred 19.6 and 50.5 h after the administration of estradiol benzoate and EC, respectively (Sales et al., 2012). This delay in EC-treated cows can be explained by EC's distinct pharmacokinetics. Estradiol cypionate has a low solubility in water and a slow release from the injection site, which prolongs the plasma concentrations of estradiol (Burton et al., 1990; Vynckier et al., 1990), delaying the follicular wave emergence and reducing the mean follicle diameter at the end of treatment with progesterone (Thundathil et al., 1998; Colazo et al., 2003; Haughian et al., 2004) or progestagens (Sá Filho et al., 2009).

In the current study, although the EC treatment may have resulted in delayed time to follicular wave emergence, the combined treatment of MGA plus EC resulted in a satisfactory service rate in the first 10 d of breeding period and acceptable pregnancy rates after AI. Similar results have also been reported for prepubertal and pubertal (Gonzalez-Padilla et al., 1975) or only for prepubertal (Anderson et al., 1996) *Bos taurus* heifers that were treated with norgestomet ear implants over a 10-d period. Long-term treatment with MGA (i.e., approximately 14 d) mimics luteal phase synchronization of estrous cycle among pubertal heifers (Patterson et al., 1989) but also induces puberty in prepubertal heifers

Table 3. Reproductive parameters according to BW classes at the onset of the breeding period of the *Bos indicus* beef heifers

Parameter	BW class, kg Light (<275)	Moderate (275–300)	Heavy (>300)	*P*-value
No. of animals	95	110	163	–
BW	265.1 ± 0.9	286.8 ± 0.6	319.9 ± 1.4	–
BCS at Day –24 of the trial, 1–5 scale	3.0 ± 0.3[2]	3.1 ± 0.3[2]	3.2 ± 0.3[1]	<0.01
Interval from onset of breeding period to estrus detection,[1] d	6.1 ± 0.5	6.6 ± 0.3	6.8 ± 0.3	0.14
Service rate,[2] %	45.3	51.8	66.9	0.13
Conception rate,[3] %	48.8	66.7	59.6	0.22
AI pregnancy rate,[4] %	21.5	34.0	40.0	0.17
Cyclicity at Day 40 of breeding period, %	82.8[2]	87.7[2]	98.7[1]	0.04
Pregnancy rate at end of breeding, %	55.8[2]	70.0[2]	87.7[1]	<0.01
Median days to pregnancy	52.2 ± 3.2[1]	41.4 ± 3.1[2]	33.2 ± 2.1[2]	0.003

[1]Estrus was detected twice daily during first 10 d of the breeding period.

[2]Service rate = number of inseminated heifers by number of heifers enrolled in the trial.

[3]Conception rate = number of pregnant heifers by number of heifers inseminated during the first 10 d of the breeding period.

[4]AI pregnancy rate = number of heifers that became pregnant following AI by total of heifers enrolled in the trial.

(Imwalle et al., 1998). The ability of progestin supplementation to increase the frequency and amplitude of LH secretion may explain its effect of hastening the occurrence of puberty (Anderson et al., 1996; Perry et al., 2005). Therefore, the use of MGA supplementation could be considered an important hormonal strategy for synchronizing the estrus of pubertal heifers and inducing the cyclicity of prepubertal *Bos indicus* heifers. Therefore, MGA potentially could have an enhancing effect in the proportion of heifers available for breeding early in the breeding period.

In Exp. 2, heifers treated with MGA had a greater service rate during the first 10 d of the breeding period than non-MGA-treated heifers. Moreover, this positive effect was also observed when considering only prepubertal heifers (MGA = 61.9% vs. no MGA = 31.2%). Positive effects of hormonal therapies on the hastening of the puberty of prepubertal heifers have been described for intravaginal progesterone devices (Tauck et al., 2007; Claro-Júnior et al., 2010) and norgestomet ear implants (Anderson et al., 1996; Hall et al., 1997) and following oral supplementation with MGA (Imwalle et al., 1998; Wood-Follis et al., 2004; Tauck et al., 2007).

Despite the greater service rate, a lower AI conception rate was achieved in the MGA-treated females compared with the no-MGA group. Remarkably, the reduced fertility at estrus immediately after treatment did not compromise the subsequent estrus or the pregnancy rates at the end of the breeding period. However, such negative effect of long-term MGA treatment on the subsequent AI has been previously described (Patterson et al., 1989). The long-term MGA treatment has been effective to synchronize estrus in 60 to 100% of treated animals. However, the rate of pregnancy throughout the breeding period can be 40 to 50% lower than no MGA. This reduced fertility of heifers that have received long-term treatment with progestin has been associated with extended interovulatory intervals and the subsequent formation of persistent DF (Patterson et al., 1989; Savio et al., 1993; Stock and Fortune, 1993; Ahmad et al., 1995; Kinder et al., 1996; Revah and Butler, 1996; Fortune and Rivera, 1999; Bridges and Fortune, 2003). The administration of MGA inhibits the preovulatory LH surge and ovulation but, similarly to progesterone derived from the CL during diestrus, it does not modulate LH pulse frequency (Kojima et al., 1992; Imwalle et al., 1998, 2002).

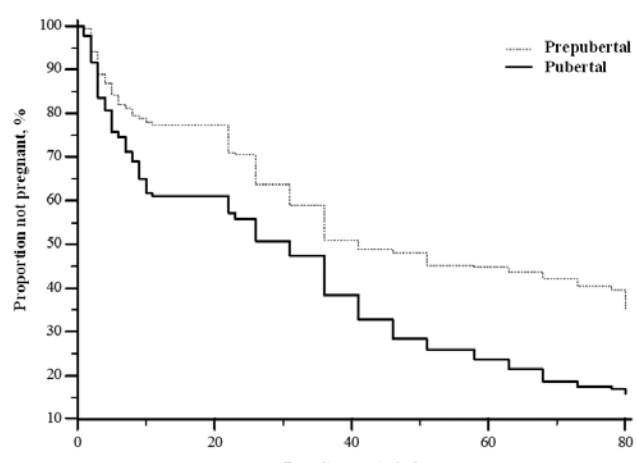

Figure 5. Survival curves for proportion of nonpregnant heifers by Day 80 of the breeding season for the heifers that were classified as prepubertal (dashed line; $n = 190$) and pubertal (solid line; $n = 178$) at beginning of the breeding period. The median intervals to pregnancy for the prepubertal and pubertal heifers were 41 and 31 d (adjusted hazard ratio = 1.40; 95% confidence interval = 1.09 to 1.82), respectively.

Intriguingly, it was observed (in Exp. 1) that the length of ovulatory follicular wave of zebu heifers was longer (11.3 ± 0.7 d) than normally reported for *Bos indicus* breeds. During the normal estrous cycle, Nelore cows and heifers typically present 7.9 d of duration of follicular dominance, similar to other breeds such as Gir cows (7.7 d) and Thai Native heifers (±7.0 d; Figueiredo et al., 1997; Viana et al., 2004; Sakhong, 2011). Furthermore, several studies have demonstrated that the duration of dominance before ovulation for optimum fertility is less than 8 d (Kinder et al., 1996; Fortune and Rivera, 1999; Bridges and Fortune, 2003). Greater conception rate was achieved when the period of dominance was restricted to 1 to 4 d, whereas dominance of >10 d was associated with decreased fertility (Ahmad et al., 1995; Revah and Butler, 1996). In agreement, the mean emergence–ovulation interval was approximately 1 d shorter in cows that became pregnant than in nonpregnant cows (Bleach, 2004). Therefore, the reduced fertility of the *Bos indicus* heifers that were inseminated on estrus detection immediately after the MGA supplementation observed in the present study potentially may have been explained by the formation of persistent follicles and the ovulation of aged compromised oocytes.

The pubertal heifers exhibited significantly increased pregnancy rates throughout the breeding period than did prepubertal heifers. This early maturation of the heifers has tremendous effects on reproductive performance (Patterson et al., 1992; Stevenson et al., 2008) but also influences the likelihood of early calving patterns in subsequent years, which reduces the risk of involuntary culling (Rhodes et al., 2003). Similarly, the *Bos indicus* heifers with greater BW (>300 kg) at the onset of the breeding period exhibited higher cyclicity and a higher rate of pregnancy during the breeding period. The effect of body growth on the reproduction of replacement heifers has been well recognized (Patterson et al., 1992; Bagley, 1993; Bossis et al., 1999; Walsh et al., 2012); however, the mechanisms that link body energy reserves and nutrition with reproductive development are not completely understood. Many studies indicate that there are direct effects of individual nutrients on the hypothalamic–pituitary–ovarian uterine axis (McShane et al., 1992; Schillo, 1992; Bagley, 1993; Ahima et al., 2000; Barb and Kraeling, 2004; Schneider, 2004; Pineda et al., 2010; Amstalden et al., 2011). Therefore, postweaning nutrition management is essential for improving the percentage of heifers that are pubertal at the onset of the breeding period. Moreover, feeding strategies should try to ensure that replacement heifers reach approximately 60 to 70% of their mature live weight at the onset of the breeding period. This target should be 300 kg BW in Nelore (*Bos indicus*), and it will likely optimize reproductive efficiency.

In conclusion, oral supplementation with MGA added to the mineral mixture before the onset of the breeding period increased the AI service rate; however, this supplementation did not alter the rate of pregnancy throughout the breeding period of pasture-grazed *Bos indicus* beef heifers. These results were independent of pubertal status at the beginning of progestin treatment. Heifers that were cycling (had already reached puberty) and those with BW over 300 kg at the onset of the breeding period exhibited greater reproductive efficiency, as indicated by greater pregnancy rate throughout the breeding period, than did heifers that were classified as prepubertal or had BW under 300 kg.

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