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Estrous response and AI pregnancy rates of 2-year-old beef heifers exposed to androgenized steers before an estrous synchronization protocol with double injection of PGF2alpha

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Abstract

The objective of this study was to determine if exposure of 2-year-old beef heifers to androgenized steers (AS) for 30 days before the beginning of an estrous synchronization protocol (ESP) and artificial insemination (AI) increases the estrous response and pregnancy rate. Hereford x Braford heifers were stratified by body weight and presence of a corpus luteum (CL) and assigned to be 1) exposed to AS (EAS; n = 53) or 2) not exposed to AS (NE; n = 53) for 30 days (Day 0 = first day of exposure). Proportions of heifers with a CL on Day -7 were 20.6 and 24.5% for EAS and NE, respectively. Ovaries of each heifer were scanned on Days 15 and 30 of the exposure period for a CL. On Day 30, steers were removed from the EAS heifers and two doses of a PGF2alpha analogue were administered to each heifer 11 days apart. Heifers were observed for behavioral estrus for 6 days, beginning 48 h after the last PGF2alpha injection, and inseminated 12 h after estrus detection. More EAS than NE heifers had a CL on Day 15 (54.7 and 32.1%, EAS and NE, respectively; P < 0.02), but there was no difference in the proportion of heifers that had a CL by Day 30 (73.6 and 72.0%, EAS and NE, respectively; P = 0.2). There were no differences between EAS and NE heifers for proportions that showed estrus (66.0 and 67.9%, EAS and NE, respectively; P = 0.8), conception rates (83.0 and 92.0%, EAS and NE, respectively; P = 0.3) and pregnancy rates (83.0 and 86.5%, EAS and NE, respectively; P = 0.6). Exposing 2-year-old heifers to AS under the conditions of this experiment before an ESP that included a double injection of PGF2alpha had no effects on estrous response and AI pregnancy rates.

Keywords: AI pregnancy rates, beef heifers, biostimulation, estrous synchronization.

Introduction

Biostimulation (male or bull effect) is an effective alternative to reduce anestrous periods in beef cattle (Berardinelli and Joshi, 2005; Oliveira *et al.*, 2009; Tauck *et al.*, 2010). Induction of ovulatory activity in females is stimulated by exposure to males through different signals, including chemical, tactile, auditory and visual signals. The response of heifers to male exposure appears to depend on body weight and age at which heifers are exposed (Fiol *et al.*, 2010) and to body growth during exposure period (Izard and Vandenbergh, 1982; Roberson *et al.*, 1991; Rekwot *et al.*, 2000).

Exposure of beef heifers to androgenized steers (Ungerfeld, 2009) or to vasectomized bulls (Oliveira *et al.*, 2009) before the breeding period increased natural-service pregnancy rates. Other studies also indicated that AI pregnancy rates may be improved by exposing beef heifers (Roberson *et al.*, 1991; Quadros and Lobato, 2004) and primiparous cows (Fernandez *et al.*, 1993) to bulls before the breeding season. Increased pregnancy rates reported in some of the aforementioned studies may have been a consequence of a greater proportion of exposed females that were cycling at the beginning of the natural service or AI breeding periods (Short *et al.*, 1990). Furthermore, variation in pregnancy rates in heifers exposed to bulls or androgenized steers may be related to age (Quadros and Lobato, 2004) and to body weight at the time of exposure (Ungerfeld, 2009). Age and body weight at the beginning of the exposure period influenced the response to males: positive and significant differences on pregnancy rates were limited to older (Quadros and Lobato, 2004) and heavier (Ungerfeld, 2009) heifers.

Estrous synchronization protocols are an effective way to reduce AI costs, and allow fixed-timed AI (Holm *et al.*, 2008). Results for AI pregnancy rates in heifers and postpartum cows exposed to bulls before or during an estrous synchronization protocol are equivocal. Exposure of postpartum beef cows to bulls or excretory products of bulls before or during (Anderson *et al.*, 2002; Berardinelli *et al.*, 2007) an estrous synchronization protocol increased fixed-timed AI pregnancy rates. Similarly, Small *et al.* (2000) using an estrous synchronization protocol that included two doses of PGF2alpha reported a 30% increase in timed AI pregnancy rates in winter-born, but not spring-born heifers exposed to bulls before the beginning of the estrous synchronization protocol compared to isolated heifers. In contrast, exposure of beef heifers to androgenized steers for 20 days (Ungerfeld, 2010) and

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postpartum cows to epididectomized bulls for 60 days (Berardinelli *et al.*, 2001) before an estrous synchronization protocol and fixed-timed AI had no benefits on conception or pregnancy rates. Considering all this information, the objective of the present study was to determine if exposure of 2-year-old beef heifers to androgenized steers (AS) for 30 days before an estrous synchronization protocol (ESP) based on PGF2alpha analogue would increase the estrous response and AI pregnancy and conception rates. The null hypotheses was that exposure of 2-year-old beef heifers to AS for 30 days before an ESP with PGF2alpha and AI would not alter the estrous response and AI conception and pregnancy rates.

Materials and Methods

Location, animals and management

The experiment was performed from October to November 2008 on a commercial farm in Rocha, Uruguay (34º S and 55º W), with 106 Hereford x Braford heifers (22-month-old, 282.5 ± 25.6 kg; mean ± SD; range = 235.5 to 371.5 kg), and 6 AS. One week before the beginning of the experiment, ovaries of each heifer were scanned (Day -7) for corpus luteum (CL) visualization using an Aloka 500 (Aloka, Tokyo, Japan) ultrasound scanner with a 5.0 MHz linear transducer. On Day 0, heifers were assigned according to body weight (BW) and presence of CL at the previous scanning to one of the following treatments: 1) exposed to AS (EAS; n = 53) for 30 days, and 2) not exposed to AS (NE; n = 53) for the 30-days experimental period. Percentages of heifers that had a CL on Day -7 for EAS and NE heifers were 20.6 (11/53) and 24.5% (13/53), respectively. Mean BW for EAS and NE heifers on Day 0 were 280.5 ± 25.0 and 284.5 ± 26.4 kg, respectively.

During the exposure period heifers in each treatment grazed native pastures in two paddocks separated by a minimum distance of 800 m, minimizing visual or olfactory contact between heifers in each treatment. On Day 15 and 30 of the experimental period, heifers were weighed and the presence or absence of a CL in the ovaries of EAS and NE heifers was determined by ultrasound. Heifers in each treatment were collected at each ultrasonographic examination, and ultrasonography was performed first in NE and then in EAS heifers to avoid any visual or olfactory contact between both groups.

Each of the 6 steers was given an i.m. injection of testosterone propionate (500 mg, Testosterona Ultralenta, Dispert, Montevideo, Uruguay) at weekly intervals beginning on Day -7 (Day 0 = start of exposure period) with the last injection on Day 21 (total = 5 injections). All AS displayed male sexual behavior at Day 0 and throughout the course of exposure period.

Estrous synchronization protocol and AI

Androgenized steers were removed from the paddock housing EAS heifers at the end of the exposure period (Day 30). Exposed and non-exposed heifers remained in the separated paddocks during the ESP. Two doses of a PGF2alpha analogue (8 µg Delprostenate, Glandinex, Universal Lab, Montevideo, Uruguay) were administered to each heifer 11 days apart, beginning on Day 30. Starting 48 h after the second PGF2alpha injection, heifers were observed visually for signs of behavioral estrus twice daily for 40 min over a 6-day interval. Heifers that displayed estrus were inseminated by AI 12 h after estrus using frozen tested semen. After Day 48, both groups were joined and remained with four fertile bulls during 2 months. Conception (number of respondents pregnant to AI/number respondents or inseminated heifers) and pregnancy rates (number pregnant/total number of heifers) were determined by ultrasound 70 days after the beginning of AI. As bulls were not introduced immediately, pregnancies resulting from the synchronized estrus were easily discriminated according to the fetus size.

Statistical analyses

Proportions of EAS and NE heifers that exhibited a CL on Day 15 and 30 and that displayed estrus during the 6 days after the second PGF2alpha injection, were analyzed using separated chi-square analyses. Likewise, conception and pregnancy rates 70 days after AI for EAS and NE heifers were analyzed by separate chi-square analyses. Pregnancy and conception rates of EAS and NE heifers that had or did not have a CL on their ovaries at Day -7 and at the beginning of the ESP were also analyzed by chi-square analysis. Body weights of EAS and NE on Day 0, 15 and 30 were analyzed with an ANOVA for repeated measures. The model included the fixed effects of treatment (EAS and NE), period (Day 0, 15 and 30) and the interaction between treatment and period. Only P-values of 0.05 or less were considered to be significantly different.

Results

Body weight was only affected by time ($P < 0.01$); it increased during the experimental period. On Day 15 heifers EAS were 323.6 ± 25.2 kg and NE were 328.1 ± 27.9 kg, while on Day 30 heifers EAS were 340.2 ± 23.9 kg and NE were 349.8 ± 26.2 kg.

More ($P < 0.02$) EAS (29/53) than NE (17/53) heifers had CL 15 days after the start of exposure to AS. However, cumulative proportion of EAS (39/53) and NE (38/53) heifers with CL 30 days after exposure did not differ.

No differences were found in estrus response after PGF2alpha, or in the conception or total pregnancy rates (females pregnant to the AI plus females pregnant to bulls) among EAS and NE that had or did not have a CL in their ovaries at the beginning of the ESP (Table 1). Similarly, proportions of EAS (35/53) and NE (36/53) heifers that displayed estrus within 6 days after the second PGF2alpha injection did not differ. Likewise, there were no differences in AI total pregnancy rates and conception rates between EAS and NE heifers (Table 2).

Among heifers with no CL on Day -7, more EAS tended to have a CL on Day 15 than NE (20/42 *vs.* 12/40, EAS and NE, respectively; P = 0.10), but no differences were observed on Day 30 (28/42 *vs*. 25/40, EAS and NE, respectively). There were no differences in estrous response (25/42 *vs*. 26/40, EAS and NE, respectively), conception (23/25 *vs*. 23/26, EAS and NE, respectively) or pregnancy (36/41 *vs*. 33/39, EAS and NE, respectively) rates between EAS and NE without CL on Day -7.

Table 1. Proportion of estrous response, and conception and pregnancy rates in beef heifers in which a corpus luteum (CL) was or was not observed during the exposing period.

	With CL			Without CL		
	EAS (%)	NE (%)	P-value	EAS (%)	NE (%)	P-value
Estrus	30/39 (77)	30/38 (79)	0.8	6/14 (36)	6/15 (40)	0.9
Conception rate[1]	24/30 (80)	27/30 (90)	0.3	6/6 (100)	6/6 (100)	1
Pregnancy rate[2]	30/39 (77)	33/38 (87)	0.3	13/14 (93)	11/14 (78.5)[†]	0.5

[†]One animal was not diagnosed.
[1]Conception rate: number of respondents pregnant to IA/number inseminated.
[2]Pregnancy rate: number of pregnant heifers/total number of heifers.
EAS: heifers exposed to androgenized steers during 30 days prior to the beginning of an estrous synchronization protocol and artificial insemination; NE (not exposed): heifers isolated from males.

Table 2. Conception and pregnancy rates in 22-month-old crossbred, beef heifers exposed (EAS) and isolated (NE) from androgenized steers for 30 days before the beginning of an estrous synchronization protocol that include two PGF2alpha injections given 11 days apart and artificial insemination.

	EAS (%)	NE (%)	P-value
Conception rate[1]	29/35 (83)	33/36 (92)	0.3
Pregnancy rate[2]	43/52 (83)[†]	45/52 (86.5)[†]	0.6

[†]One animal was not diagnosed.
[1]Conception rate: number of respondents pregnant to IA/number inseminated.
[2]Pregnancy rate: number of pregnant heifers/total number of heifers.

Discussion

Exposure of beef heifers to AS increased the proportion of heifers that had a CL 15 days after the beginning of the exposure period. Among heifers without a CL at Day -7, presence of CL on Day 15 tended to be greater in heifers exposed to AS than in non-exposed heifers. Although CL observations were performed with 15 day intervals, the difference observed suggests that there was a short-timed advance in exposed heifers, as 15 days later no differences in ovulatory activity between exposed and isolated heifers were observed. Positive effects of male exposure on onset of puberty was related to age (Quadros and Lobato, 2004; Fiol *et al.*, 2010) and body weight (Fiol *et al.*, 2010) at the beginning of the exposure period, and to body growth during male exposure (Izard and Vandenbergh, 1982; Roberson *et al.*, 1991; Rekwot *et al.*, 2000). Moreover, Small *et al.* (2000) determined that efficacy of bull exposure on cyclic activity in heifers was dependent upon the proximity of the heifers to the spontaneous attainment of puberty when stimulatory treatments are applied. In addition, in postpartum beef cows, positive effect of male exposure on cyclic activity was related to nutritional status at the beginning and during the exposure period: male presence was effective to reduce anestrous period among cows in moderate nutritional status, but had no effects on cows in high nutritional status (Monje *et al.*, 1992; Stumpf *et al.*, 1992). In our experiment, all of the heifers had a good nutritional status during the experimental period, so it is possible to speculate that, according to their age and body weight, they were near their spontaneous onset of cyclic activity (Quintans *et al.*, 2007). Therefore, the lack of differences in the proportion of cyclic heifers at the end of the exposure period may be due to the natural onset of puberty in an important proportion of animals.

In the present experiment, previous exposure to AS did not affect the proportion of heifers that exhibited estrus after the second PGF2alpha injection in an ESP that included 2 injections of PGF2alpha given 11 days apart. This result is consistent with previous studies in which biostimulation before or during an ESP (Anderson *et al.*, 2002; Berardinelli *et al.*, 2007; Tauck and Berardinelli, 2007), or before a natural service (Assis *et al.*, 2000) did not appear to influence the estrous response in heifers.

In agreement with results reported by Ungerfeld (2010) and Berardinelli *et al.* (2001), neither pregnancy nor conception rates differed between EAS and NE. In the study of Ungerfeld (2010), beef heifers and postpartum cows were exposed to AS for 15 days before the beginning of an ESP with progestins plus fixed-time AI. Cyclic activity at the beginning of the exposure period was not recorded in that experiment, but Berardinelli *et al.* (2001) reported that cyclic activity was greater at the beginning of an ESP in postpartum cows exposed to males during an ESP with GnRH and PGF2alpha, than in non-exposed heifers, but no differences on pregnancy rates were recorded. In contrast, others reported that exposure to males or excretory products of males during an ESP based on GnRH and PGF2alpha increased the proportion of cyclic females at the beginning of the ESP and conception rates to timed AI (Anderson *et al.*, 2002; Berardinelli *et al.*, 2007; Tauck and Berardinelli, 2007). In prepubertal, winter-born beef heifers, exposure to bulls before an ESP with double PGF2alpha and fixed-timed AI, advanced the onset of puberty and increased pregnancy rates in exposed heifers (Small *et al.*, 2000). In agreement, exposure of heifers to vasectomized bulls for 50 days (Quadros and Lobato, 2004) and 175 days (Roberson *et al.*, 1991) before AI plus natural service increased conception rates of prepubertal beef heifers. In both of those studies (Roberson *et al.*, 1991; Quadros and Lobato, 2004), the proportion of exposed heifers that had exhibited cyclic activity was greater than that for heifers not exposed to males, and might explain the differences in pregnancy rates between exposed and non-exposed females. In natural service conditions, pregnancy rates were greater for heifers exposed to AS during only 15 days (Ungerfeld, 2009) and for prepuberal heifers exposed to sterilized bulls for 210 days (Oliveira *et al*., 2009), compared to those isolated from males. Therefore, more exposed females cycling at the beginning of the ESP seem to be necessary to obtain positive effects of biostimulation on breeding performance of females exposed to males before an ESP with double PGF2alpha. Thus, it is possible that in our study, similar proportion of cyclic heifers at the beginning of an ESP along with a short exposure period, might explain the similar conception and pregnancy rates between EAS and NE.

Ovulatory activity was transitory induced in 2-year-old beef heifers exposed to AS during 15 days, but no difference was found on the proportion of heifers that had a CL at the end of the exposure period of 30 days. We did not record any positive effect after the exposure period on estrous responses and AI pregnancy rates after a double injection of PGF2alpha.

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