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Response to biostimulation in peri-puberal beef heifers: influence of male-female proximity and heifer's initial body weight

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Abstract

The objectives were to determine if exposure of 12 mo old peri-puberal beef heifers to androgenized steers for 35 d hastened puberty, and if the response was related to the physical proximity between males and females and to heifer's initial body weight. Hereford x Aberdeen Angus heifers (n = 131), 12 mo old, were assigned to two treatments: 1) Exposed group, exposed to androgenized steers from Day 0 to Day 35 (E, n = 66); or 2) Control group, isolated from the steers and other males (C, n = 65). Cyclic activity was determined through estrous behavior detection (twice daily) and weekly ultrasound imaging to detect a CL. For each Exposed heifer, an association index (with males) was determined thrice weekly, based on the distance to androgenized steers every 10 min for 4 h (until cyclic activity began). Data were analyzed according to heifer's initial body weight, which was categorized into three ranges (low, medium, and high, designated LW, MW, and HW, respectively). The cumulative proportion of cyclic heifers was greater for Exposed than Control heifers as of Day 21. By the end of the exposure period, more Exposed than Control heifers had attained puberty (16/66 vs. 2/65; $P < 0.001$). Within the HW classification, more Exposed than Control heifers became puberal (11/20 vs. 2/21; $P = 0.002$). However, there were no differences between MW and LW in the proportions of heifers that reached puberty. Association index in Exposed heifers was greater in HW than in MW and LW (0.10 ± 0.09 vs. 0.06 ± 0.03 and 0.06 ± 0.04; $P < 0.05$), and in heifers that began cyclic activity compared to those that did not in the MW heifers (0.09 ± 0.05 vs. 0.05 ± 0.02; $P = 0.01$) and tended to be different in the HW treatment (0.12 ± 0.10 vs. 0.06 ± 0.02; $P = 0.09$). In conclusion, exposure of peri-puberal beef heifers to androgenized steers for 35 d advanced puberty in heavier heifers; an earlier response occurred in heifers with greater proximity to androgenized steers.

Keywords: Male effect; Biostimulation; Puberty; Socio-sexual stimulus; Beef heifers

1. Introduction

Age at puberty is a major factor affecting reproductive performance in cattle, especially in seasonal breeding systems [1]. Early puberty is necessary to reduce age at first conception and first calving, and to achieve optimal lifetime productivity [2]. In Uruguay, beef heifers managed under grazing conditions begin cyclic activity between 15 and 17 mo of age if body weight is between 278 and 295 kg [3,4]. Although there are many strategies to advance and regulate the onset of puberty, most are based on hormonal treatments [5], whereas markets demand "clean, green, and ethical" products [6]. Therefore, the development of other alternatives, e.g., socio-sexual stimuli, emerges as an additional

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strategy to be included in farm management for hormone-free production.

The stimulatory effects of males on female cyclic activity through genital stimulation, pheromones, or other less defined external cues, is known as biostimulation [7]. In cattle, biostimulation may be provoked with bulls, testosterone-treated cows [8], or testosterone- treated steers [9]. Exposure of cows to bulls decreased postpartum anestrous interval [10–15]. However, information related to the success of biostimulation in advancing the onset of puberty in heifers, and the identification of the factors that influence the response of heifers, is scarce.

Little is known about the signals from bulls that stimulate cyclic activity in beef heifers. Burns and Spitzer [8] reported that the interval from calving to estrus in beef cows was reduced after exposure to bulls or androgenized females. In other studies, fenceline exposure to bulls accelerated the resumption of cyclic activity in primiparous postpartum, anestrus cows [16,17], but not as effectively as direct male-female contact [16]. Alvarez et al [18] reported a direct link between male-female physical proximity and the interval to ovulation when female goats were stimulated by bucks.

Nutritional status and growth rate have a direct association with age at puberty in beef heifers [3,19–21], and both appeared to be involved in the response to biostimulation. Heifers raised in mixed-gender groups reached puberty younger and at lower live weights than those isolated from males [22]. Roberson et al [23] reported that heifers that exhibited high rates of gain during bull exposure reached puberty earlier than those that exhibited medium rates of gain. It remains to be determined if there is a relationship between initial body weight of heifers and the response to biostimulation.

In the present experiment two hypotheses were tested: 1) exposure of peri-puberal beef heifers to androgenized steers advances the onset of puberty; and 2) male-to-female proximity and initial body weight of the heifers influences the response to biostimulation. Therefore, the first objective of this study was to determine if exposure of 12 mo old peri-puberal beef heifers to androgenized steers for 35 d advances the onset of ovarian cyclic activity. Two complementary aims were to determine: 1) if male-to-female proximity influences the response to biostimulation; and 2) if there is a relationship between initial body weight of heifers and the response to biostimulation.

2. Materials and methods

2.1. Animals, housing, and treatments

The study was performed from October to December in the Experimental Unit "Palo a Pique", INIA Treinta y Tres, Uruguay (34° S and 55° W), with 131 Hereford x Aberdeen Angus heifers. At the beginning of the experiment, heifers were (mean ± SD) 12.1 ± 3.4 mo old (range, 11.0–14.2) and weighed 226.0 ± 28.2 kg (range, 168.5–300.0). Groups of heifers were blocked according to age and body weight, and assigned to two treatments: 1) Exposed group: exposed to androgenized steers from Day 0 to Day 35 (E, n = 66); and 2) Control group: isolated from males (C, n = 65). Both groups of cattle grazed native pastures in two separate paddocks located at a minimum distance of 1000 m of each other, so that Control heifers could not see or smell the males. On Day 35, steers were removed from the Exposed group, and all heifers were grouped and managed together for 1 wk in the paddock where Control heifers were initially located. Four steers received testosterone propionate (800 mg IM, Testosterona Ultra Fuerte, Dispert, Montevideo, Uruguay) once a week from Day −7 (with Day 0 being the date in which steers were grouped with Exposed heifers) to Day 14. On Day 14 they were removed and replaced with other four steers, equally treated from Day 7 to the end of the exposure period (Day 35). During the course of the exposure period, all androgenized steers displayed male sexual behavior.

Heifers were weighed once a week from Days 0 to 42. For data analysis, heifers were categorized according to their initial body weight, establishing three body weight ranges: low-weight (LW) heifers ranged from 168 to 211 kg (E-LW, 197.0 ± 12.2 kg, n = 22; C-LW, 199.0 ± 10.2 kg, n = 22), medium-weight (MW) from 212 to 236 kg (E-MW, 223.2 ± 8.1 kg, n = 24; C-MW, 223.2 ± 7.4 kg, n = 22) and high-weight (HW) from 237 to 302 kg (E-HW, 260.0 ± 19.5 kg, n = 20; C-HW, 258.7 ± 18.8 kg, n = 21).

To determine possible influences of age on the onset of puberty, heifers were stratified according to the age at the beginning of the experiment into three categories: youngest (YH) those heifers ranged from 336 to 388 d of age (E-YH, 373.0 ± 15.3 d, n = 19; C-YH, 374.0 ± 13.5 d, n = 22), medium heifers (MH) from 389 to 402 d (E-MH, 395.2 ± 4.2 d, n = 22; C-MH, 397.0 ± 4.3 d, n = 19), and oldest heifers (OH) from 403 to 433 d (E-OH, 419.1 ± 9.0 d, n = 21; C-OH, 414.0 ± 8.4 d, n = 19).

2.2. Determination of cyclic activity

Estrous behavior was recorded twice daily for 40 min by two experienced observers during the 42 d of the experiment. Ovaries of each heifer were scanned once weekly to determine the presence of the CL using an Aloka 500 (Aloka, Tokyo, Japan) ultrasound scanner with a 5.0 MHz linear transducer. Ultrasound scanning began on Day -14 and finished on Day 42. The onset of cyclic activity was defined as the day in which standing estrus was recorded, followed by visualization of a CL, or the first date of two successive scans that a CL was observed in an ovary.

2.3. Association index (AI)

An association index (AI) [24] between each Exposed heifer with steers was used to determine the relationship of proximity with presence or absence of cyclic activity. The distance to the androgenized steers was recorded every 10 min for 4 h (9:00–11:00, and 16:00–18:00), three times per week from Days 0 to 35. Index value was 1 for the heifers that were <1 body length away from a steer, 0.33 if they were between 1 and 3 body lengths, and 0 when they were more than 3 body lengths. An AI was calculated daily for each heifer as the mean of her recordings, and total AI was determined as mean AI, considering only data collected before the onset of cyclic activity for each heifer.

2.4. Statistical analysis

Cumulative proportions of cyclic heifers were determined at 7 d intervals, and compared using a Chi-square test. Initial body weight, FBW were compared with a 2 × 3 ANOVA, with treatment (Exposed vs. Control) and the three body weight categories as main effects. The AI was compared with ANOVA after Bliss-transformation (Arcsin $\sqrt{x}$). Post-hoc comparisons were made with an LSD test.

3. Results

3.1. Onset of puberty

None of the heifers used in this study ($n = 131$) had a CL in their ovaries before the start of the exposure period (Days −14, −7, and 0). Cumulative proportions of heifers that started to cycle were greater in Exposed than in Control heifers by Day 21 of the exposure period ($P < 0.03$; Fig.1). By the end of the exposure period, more Exposed than Control heifers had attained puberty (16/66 vs. 2/65; $P < 0.001$). Age at puberty of heifers that had began their cyclic activity by the end of the experiment (Day 42; 19/66 vs. 9/65, Exposed and Control heifers, respectively) tended to be lower in Exposed than in Control heifers (428.4 ± 21.5 vs. 441.1 ± 16.0; $P = 0.06$).

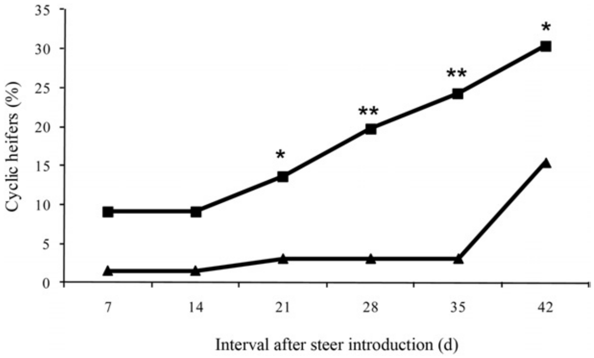

Fig. 1. Cumulative proportion (%) of cyclic beef heifers at 7 d intervals during the experiment. Exposed group (-■-): heifers exposed to androgenized steers from Day 0 to Day 35; Control group (-▲-): heifers isolated from steers throughout the experimental period. On Day 35, steers were removed and all heifers were grouped together for 1 wk.

* $P < 0.05$; ** $P < 0.01$.

Initial body weight, ADG, and FBW among treatments in the three body weight ranges are shown (Table 1). There were differences in ADG between E-MW and C-MW ($P < 0.05$) during the experimental period. Cumulative proportions of cyclic heifers for the three body weight ranges are shown (Fig. 2). Within HW, more Exposed than Control heifers began cyclic activity (11/20 vs. 2/21; $P = 0.002$). Furthermore, more E-HW than C-HW heifers were cycling on Days 21 (8/20 vs. 2/21), 28 (10/20 vs. 2/21), 35 (11/20 vs. 2/21), and 42 (14/20 vs. 7/21; $P < 0.05$; Fig. 2A). There was no difference in the proportions of Exposed and Control heifers, classified as medium or low initial body weight that attained puberty during the experiment (Figure 2B and 2C).

None of the youngest (336–388 d) Control heifers initiated cyclic activity by the end of the experiment (Fig. 3). More E-YH than C-YH began to cycle from Day 28 to the end of the experiment (D 42; $P < 0.05$; Fig. 3A). More E-OH than C-OH tended to be cyclic on Days 7 and 14 ($P = 0.087$; Fig. 3C).

3.2. Association index

Association indices for the three body weight ranges and between Exposed heifers that began cyclic activity and those that remained anestrus are shown (Table 2). Association index was greater for HW compared to

Table 1
Mean ± SD growth characteristics in beef heifers (in three body weight ranges) exposed (E) and isolated (Control, C) from androgenized steers.

Body weight range (kg)[1]	Group (No.)	Initial body weight (kg)	Average daily gain (kg/d)	Final body weight (kg)
LW	E (22)	196.7 ± 12.2	0.91 ± 0.15	241.3 ± 17.0
	C (22)	199.0 ± 10.2	0.86 ± 0.17	241.3 ± 13.4
MW	E (24)	223.2 ± 8.1	0.95 ± 0.14[a]	270.0 ± 9.0
	C (22)	223.2 ± 7.4	0.87 ± 0.16[b]	266.0 ± 9.2
HW	E (20)	260.0 ± 19.5	0.95 ± 0.19	306.3 ± 19.2
	C (21)	259.0 ± 19.0	0.89 ± 0.20	302.5 ± 23.5

[1] Body weight range: LW: Low body weight (168-211 kg; n = 44); MW: Medium body weight (212-236 kg; n = 46); and HW: High body weight (237-302 kg; n = 41).
[a,b] Means without a common superscript differed ($P < 0.05$).

MW and LW heifers ($P < 0.05$), and for heifers that began cyclic activity compared to those that did not ($P < 0.001$) by the end of the experiment. Among body weight ranges, there were differences between cyclic and non-cyclic heifers in the MW ($P < 0.01$), with a tendency ($P = 0.09$) in the HW range. Association indexes from heifers that began cycling in the three body weight ranges did not differ.

4. Discussion

The exposure of peri-puberal, 12 mo old beef heifers to androgenized steers for 35 d advanced the onset of puberty. These results supported previous findings in heifers with varying initial body weights and ages, and performed under various management conditions [23,25,26]. In the present study, heifers remained grazing native pastures during the whole experiment, were younger than those used by Quadros and Lobato [26], older than those used by Izard and Vandenbergh [25] and Roberson et al [23], and had lower initial body weights than in the above-mentioned studies.

Differences in cyclic activity between heifers exposed and isolated from males were first observed 3 wk after the exposure started. In contrast, others reported no effect of biostimulation on the onset of puberty in beef heifers, even with short-term (3 wk) [27] or long-term (152 d) [28] periods of exposure. Our results supported the hypothesis that, in heifers, periods of exposure to males necessary to obtain a positive response to biostimulation are longer than those observed in sheep and goats [reviews: 29,30]. However, positive responses to biostimulation in the present study occurred after a shorter interval than had been previously reported [23,31]. Perhaps heifers in the present study that responded positively to biostimulation were very close to the spontaneous onset of puberty, which may reduce the duration of exposure needed to obtain a positive response. In that regard, significant differences were observed only in youngest heifers. Similarly, cows exposed to bulls later in the postpartum anestrus resumed cycling activity (biostimulatory effect of bulls) more rapidly than when bull exposure occurred earlier after calving [12].

There was a sudden increase in the percentage of isolated heifers that started to cycle after joining both groups. There are two possible explanations: 1) joining occurred when non-stimulated anestrual heifers spontaneously began their cyclic activity; or 2) a female-female stimulating effect. The latter was supported by previous observations [32–34]; exposure of postpartum, anestrus cows to cervical mucus from estrual cows [32] and to the excretory products of cows [33], decreased the interval to resumption of cyclic activity. In post-puberal dairy heifers, the oronasal treatment with cervical mucus of estrous cows improved the synchrony of estrus, compared to those heifers treated with water [34]. Perhaps similar female-female effect occurred in our study by the time both treatments were joined. In addition, the increase of the sexually active group [15] during the last 7 d of the experiment and the large number of cyclic females in the exposed group may have promoted cyclic activity of Control heifers. The latter, recognized as a "female-effect", may have contributed as a secondary aid-effect.

As previously reported [26], ovulatory response to male exposure was only observed in heavier heifers, suggesting the existence of a threshold body weight necessary to respond to biostimulation. Those body weights are probably very close to the threshold necessary for spontaneous puberty achievement [19,35]. In postpartum cows, a positive relationship was reported between the percentage of cows responding to the presence of males and nutrition [36]. In contrast, Stumpf et

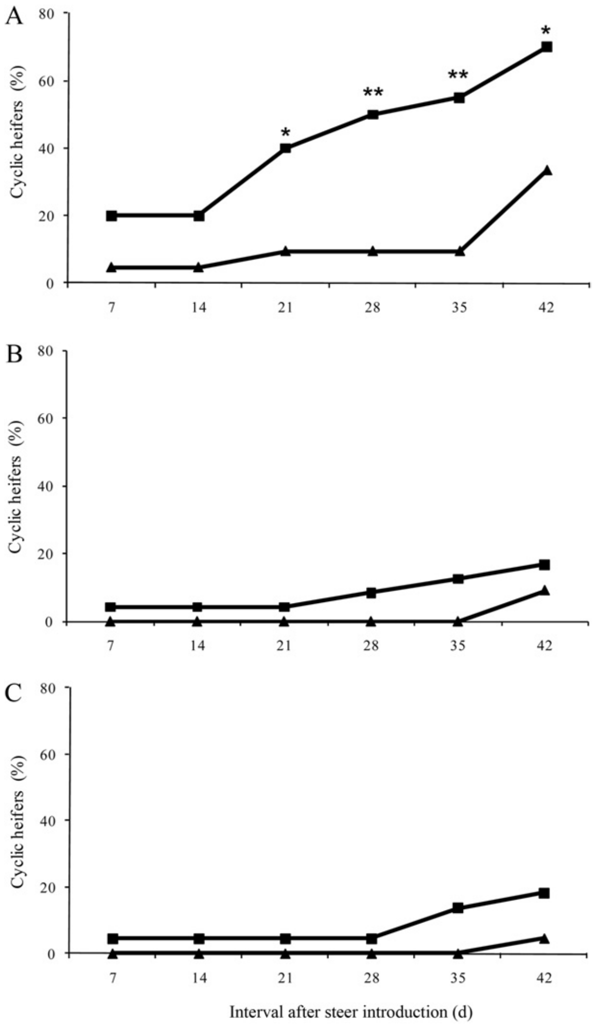

Fig. 2. Cumulative proportion (%) of cyclic beef heifers at 7 d intervals in the three body weight ranges: A) 237–302 kg; B) 212–236 kg; and C) 168–211 kg. Exposed group (-■-): heifers exposed to androgenized steers from Days 0 to 35; Control group (-▲-): heifers isolated from steers during all the experimental period. On Day 35, steers were removed and all heifers were grouped together for 1 wk.
* $P < 0.05$; ** $P < 0.01$.

al [37] reported that postpartum cows in moderate body condition were more responsive to bull biostimulation than were cows in higher body condition. Conversely, although leaner heifers in our study had high body weight gains (Table 1), this was not enough to trigger the mechanisms involved in the response. Roberson et al [23] reported a greater effect of bull exposure in heifers with high body weight gain than in heifers with medium body weight gain; however, heifers used in that study were initially heavier than those used in ours (248 kg vs. 226 kg). Overall, in the present study, the initial body weight of the heifers appeared to be a critical factor affecting the reproductive response to the presence of males, independent of the rate of body weight increase (or daily body weight gain).

The response of postpartum, anestrus cows to the biostimulatory effect of males was directly related to the nature of the intensity of the stimuli [16]. Fenceline

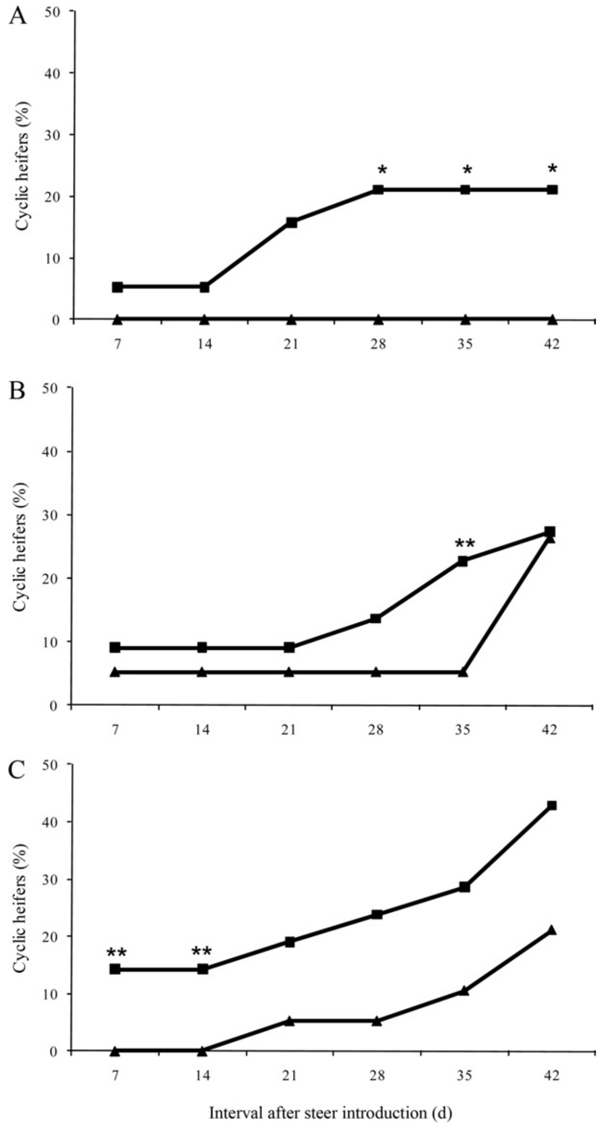

Fig. 3. Cumulative proportion (%) of cyclic beef heifers at 7 d intervals according to the age at the beginning of the experiment: A) 336–388 d; B) 389–402 d; C) 402–433 d. Exposed group (-■-): heifers exposed to androgenized steers from Day 0 to Day 35; Control group (-▲-): heifers isolated from steers during all the experimental period. On Day 35, steers were removed and all heifers were grouped together for an additional week.
* $P < 0.05$; ** $P = 0.087$.

Table 2
Mean ± SD association index (AI) in beef heifers exposed to males that began cyclic activity (Yes) and those that did not (No) by the end of the experiment, based on three body weight ranges, and mean AI for each body weight range and cyclic activity (Overall AI).

Body weight range (kg)[1]	Cyclic activity[1]		Overall AI
	Yes	No	
LW (22)	0.06 ± 0.04 (4)[A]	0.06 ± 0.03 (18)[A]	0.06 ± 0.03[a]
MW (24)	0.09 ± 0.05 (7)[A]	0.05 ± 0.02 (17)[B]	0.06 ± 0.04[a]
HW (20)	0.12 ± 0.10 (15)[A]	0.06 ± 0.02 (5)[C]	0.10 ± 0.09[b]
Overall AI	0.10 ± 0.09 (26)[A]	0.06 ± 0.02 (40)[B]	

[1] Numbers in brackets indicate heifers in each category.
[A-C] A vs. B: $P < 0.05$; A vs. C: $P = 0.09$.
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).
[1] LW: Low body weight (168–211 kg); MW: Medium body weight (212–236 kg); HW: High body weight (237–302 kg).

and intermittent contact of cows with bulls accelerated the resumption of cyclic activity [16,38], but neither were as effective as continuous and close physical contact [17,38]. In our study, AIs differed in medium and high body weight heifers that began to cycle and those that did not. This confirmed and expanded previous observations by Alvarez et al [18] in goats, who observed a positive link between the AI and the time from male introduction to ovulation. The present results supported the hypothesis that in heifers, as previously reported in postpartum cows, close physical contact between males and females is a direct determinant of the intensity of the stimulus, and thus, of the percentage of heifers responding to the presence of males. Moreover, heavier heifers were more stimulated than leaner heifers. Our study did not distinguish a preference of males for heavier heifers leading to a more intensive proceptive behavior in them. Future research could determine the relation between male and female preferences and the response to biostimulation.

We concluded that, under the conditions of this experiment, exposure of peri-puberal beef heifers to androgenized steers hastened the onset of puberty. Our results also supported the hypothesis that male-to-female physical proximity positively influenced the response, and that heifer's initial body weight at the beginning of the exposure period was directly related to the response.

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