Effect of growth rates on hormonal and pubertal status in Nellore heifers early weaned

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Abstract

This study aimed to determine the effect of growth rates on the hormonal status and puberty onset. Forty-eight Nellore heifers weaned at 3.0 ± 0.1 months old were blocked according to BW at weaning (84 ± 2 kg) and randomly assigned to treatments. The treatments were arranged in 2 x 2 factorial according to the feeding program. The first program was high (H; 0.79 kg/day) or control (C; 0.45 kg/day) ADG from 3rd to 7th month of age (growing phase I). The second program was also high (H; 0.70 kg/day) or control (C; 0.50 kg/day) ADG from the 7th month until puberty (growing phase II), resulting in four treatments: HH (n = 13), HC (n = 10), CH (n = 13) and CC (n = 12). To achieve desired gains, heifers in high ADG program were fed *ad libitum* dry matter intake (DMI), and the control group was offered around 50% of *ad libitum* DMI of high group. All heifers received a diet with similar composition. Puberty was assessed weekly by ultrasound examination, and the largest follicle diameter was evaluated every month. Blood samples were collected monthly to quantify leptin, IGF1, and LH. At 7 months of age, heifers in high ADG were 35 kg heavier than the control. Heifers in the HH had greater DMI compared with CH in phase II. The puberty rate at 19 months old was greater in the HH treatment (84%) than in the CC (23%), but there was no difference between HC (60%) and CH (50%) treatments. Heifers from HH treatment had greater serum leptin concentration than others at 13 months old, and serum leptin was greater in HH compared with CH and CC at 18 months old. High heifers in phase I had greater serum IGF1 concentration than the control. In addition, HH heifers had a greater diameter of the largest follicle than CC. There was no interaction between phases and age in any variable relative to the luteinizing hormone (LH) profile. However, the heifers` age was the main factor that increased the frequency of LH pulse. In conclusion, increasing ADG was associated with greater ADG, serum leptin and IGF-1 concentration, and puberty onset; however, LH concentration was affected mainly by age of the animal. The increasing growth rate at younger age made heifers more efficient.

Introduction

Several studies have shown the effect of metabolic imprinting in heifers induced by increases in metabolic status around 3rd to 7th month of age, which accelerate the age at puberty (Gasser et al 2006a; Cardoso et al., 2014; Moriel et al. 2014). Previous studies have reported that feeding energy-dense diets associated with early weaning advanced ovarian maturity in heifers, increased the maximum diameter of dominant follicles, the length of follicular wave (Gasser et al. 2006b), and estradiol peak during follicular wave (Gasser et al. 2006c). Therefore, associating energy dense diets with early weaning may be an efficient nutritional strategy to eliminate the static phase during the prepubertal phase, anticipating the puberty of beef heifers (Gasser et al. 2006d). However, most of the studies assessing nutrition's effect on heifers' puberty were conducted using Taurine breeds. Therefore, the effect of these strategies on Nellore heifers, which may be considered a non-precocious breed, is unknown.

Although, it is common that Nellore heifers to reach puberty only after 24 months old (Nepomuceno et al. 2017; Ferraz Junior et al. 2018). Previous studies have reported that Nellore heifers were considered precocious when heifers reached puberty earlier than 18 months old (Nepomuceno et al. 2017; Ferraz Junior et al. 2018). Whereas, a precocious Angus heifer was considered precocious when puberty occurred earlier than 10 months old (Gasser et al. 2006b; Gasser et al. 2006c; Gasser et al. 2006d). Therefore, there is the need to develop strategies to advance puberty in Nellore heifers. Moreover, it unknown how and when greater growth rates are more important in Nellore heifers prior to reach puberty. Therefore, the objective of the current study was to evaluate the effect of different growth rates by programmed feeding on the hormonal and puberty status of Nellore heifers.

Material And Methods

The experiment was carried out at the Laboratory of Animal Nutrition and Reproduction (LNRA), Department of Animal Science, "Luiz de Queiroz" College of Agriculture - ESALQ/USP, Piracicaba, SP, Brazil. The Animal Care and Use Committee from the University of São Paulo approved all animal procedures (# 7595290414).

Animals and experimental design

Forty-eight Nellore heifers weaned at 3.0 ± 0.1 months old, daughters from the same sire with small expected progeny difference (EPD within 10% percentile) for age at first calving according to Gensys (2013) catalog were used. Heifers were blocked according to initial BW at weaning (84 ± 2 kg) and randomly assigned to the treatments. The treatments were arranged in 2 x 2 factorial with two feeding programs. The first was high (H) or control (C) ADG from 3rd to 7th months old (growing phase I). The second was also high (H) or control (C) ADG from 7th months old to puberty (growing phase II), resulting in 4 different feeding treatments (HH, n = 13; HC, n = 10, CH, n = 13; CC, n = 12). To achieve desire ADG, heifers were fed at *ad libitum* dry matter intake (DMI) in high ADG and around 50% of *ad libitum* DMI in control group.

All heifers received the same diet formulated to supply 19% and 14% of CP in the first and second phases, respectively. Ingredients and chemical analyses of the experimental diets are shown in Table 1. Heifers were housed in collective pens (3 heifers/pen in *ad libitum* DMI and 2 heifers/pen in restricted DMI) and fed once a day using a forage wagon equipped with an electronic scale (Totalmix® 1500, Casale, São Carlos, SP, Brazil). Body weight was measured weekly from all heifers (Beckhauser idBeck 3.0®, Beckhauser, Paranavaí, PR, Brazil). After each BW measurement and individual ADG analysis, heifers within treatments were regrouped if the ADG difference in the same pen was greater than 0.10 kg/d. The amount of feed offered to each pen was adjusted weekly based on ADG from the previous weeks. Diet samples were collected for chemical composition analysis, and the orts were weighed for DMI calculation. The experimental diets were formulated according to the NRC (1996) to promote an ADG of 1.0 kg/d in the *ad libitum* fed group. Diet total digestible nutrients (TDN) were calculated based on the NRC (1996). Feed efficiency was determined as the ratio between ADG and DMI.

Table 1
Ingredients e chemical composition of experimental diets (DM basis).

Item	Phase I [a]	Phase II [b]
Ingredients [c] (% of DM)		
Cynodon haylage	21.5	-
Sugarcane bagasse	-	19.6
Whole cottonseed	-	10.1
Ground corn	57.5	59.1
Soybean meal	21.0	10.7
Urea	-	0.5
Analyzed chemical composition (% of DM)		
Dry Matter (%, as fed basis)	78.6	75.5
Crude Protein	19.5	14.1
Ether extract	4.3	5.9
Neutral Detergent Fiber	25.2	23.6
Ash	5.6	4.2
Total Digestible Nutrients [d]	79.1	78.7
[a] Phase I: from 3rd to 7th month of age.		
[b] Phase II: from 7th to puberty or 19th month of age in non-pubertal heifers.		
[c] Mineral supplement was provided in mineral boxes *ad libitum*, and 30 ppm of sodium monensin (Rumensin 100®, Elanco Animal Health, São Paulo, SP, Brazil) was added to the diet.		
[d] Based on the tabular composition of ingredients (NRC 1996)		

Chemical analysis and calculations

Samples of the diets and orts were ground in a Wiley mill (Marconi, Piracicaba, SP, Brazil) with a 1.0 mm sieve. The dry matter (DM) was determined after oven-drying the samples at 105°C for 24 hours according to the method of the Association of Official Analytical Chemist (#934.01) (AOAC 1990). Ash was determined by incinerating the samples in a muffle furnace at 550°C for 4 h (AOAC 1990; #942.05). Total nitrogen (N) concentration was determined using the Leco Tru Mac® /N (Leco® Corporation, St. Joseph, MI, USA; AOAC, #968.06) (AOAC 1990). Crude protein (CP) was obtained by multiplying the total N content by 6.25. Neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991), using thermostable alpha-amylase and sodium sulfite. Acid detergent fiber (ADF) was determined according to AOAC (#954.01) (AOAC 1990) using the Ankon 2000® Fiber Analyzer (Ankom Technology Corporation, Fairport, NY, USA). The ether extract (EE) content was determined using a supercritical fluid extraction system (Leco TFE-2000®, Leco Corporation, St. Joseph, MI, EUA).

Ultrasonography exam, sampling, and hormonal assay

All heifers were submitted to weekly transrectal ultrasonography (US) exam (DP-2200 VET; Mindray, Shenzhen, China) to evaluate the largest diameter of dominate follicle and corpus luteum once they reach 230 kg of BW. The largest follicle diameter was assessed monthly by US exam every 2 days for 15 days. Moreover, blood samples were collected to determine circulating concentrations of progesterone (P4) if a corpus luteum (CL) was detected. Heifers were considered pubertal when a CL was detected by the US and confirmed by progesterone concentrations greater than 1.5 ng/mL (Cooke and Arthington 2009). Blood samples were also collected to determine the leptin and IGF1 concentrations at 3, 5, 7, 12, and 18 months old. Heifers were removed from the experiment as they reach puberty or at the 19th month of age when the experiment was finished. At 19 months, non-pubertal heifers received an intravaginal device of progesterone (1,9 g) previously used for 21 days. After 9 days, the device was removed, and 7 days later, the presence of CL on ovaries was checked via US.

All blood samples for P4, leptin, and IGF1 determinations were collected from the coccygeal vein or artery in Vacutainer tubes (Greiner Bio-One Brasil, Americana, SP, Brazil) with inert serum separator gel. Serial blood from 5 heifers per treatment was collected at 15-minute intervals for 12 hours to assess luteinizing hormone (LH) pulsatility at 9, 11, 13, 15, 16, 17, 18, and 19 months old. These samples were collected via indwelling jugular catheters, and blood was allowed to clot for 24 to 48 h at 4°C. All blood samples collected were centrifuged for 15 min at 1,800 × *g* (Refrigerated Centrifuge Excelsa®4, Mod.280R – Fanem®, São Paulo, SP, Brazil), and the harvested serum was frozen at − 20°C until further analysis.

The P4 concentrations were determined by a chemiluminescent assay using commercial IMMULITE® 1000 kits (Siemens Healthcare Diagnostics Products, Llanberis, United Kingdom). The P4 analysis sensitivity was 0.002 ng/mL, and the coefficient of variation (CV) for the high and low adjustments were 1.7 and 2.1%, respectively. Leptin concentrations were evaluated by a commercial radioimmunoassay kit (Multi-Species Leptin®, Millipore – XL – 85k, Bedford, MA,

USA), as reported previously (Ren et al. 2002). The intra- and inter-assay CV were 10.3 and 7.4%, respectively, and the sensitivity of the assay was 0.955 ng/mL.

As previously described, LH concentrations were determined in duplicate using radioimmunoassay (Bolt et al. 1990; Bolt and Rollins 1983). A highly purified LH (AFP8614B; National Hormone and Pituitary Program) was used for the iodinated tracer and reference standard preparation. The sensitivity of the assay was 0.05 ng/mL. The intra- and inter-assay coefficients of variation were 5.3% and 13.9%, respectively. The LH pulse, LH pulse amplitude, and mean LH concentrations were determined as described by Goodman and Karsch (1980). Heifers were removed from blood sampling procedures when ovulation was confirmed.

Statistical analysis

Heifers were used as experimental units to evaluate the effects of the treatment on the pubertal status and hormonal concentrations. The pens were used as the experimental units to evaluate the effect of treatments on growth performance data. The continuous variables were analyzed for normality (Shapiro-Wilk) and homogeneity of variance (Welch test) before analysis with the Mixed procedure of SAS (version 9.3; SAS Institute, Cary, NC, USA), and Satterthwaite approximation was used to determine the denominator degrees of freedom for the treatment effect. The Mixed procedure also evaluated the leptin, IGF1, and LH concentrations using repeated measures. In addition, another model was constructed to evaluate the effect of puberty status (pubertal and non-pubertal heifers) on LH profile and the largest follicle diameter. The percentage of pubertal heifers at the 19th month of age and the responses to the puberty induction protocol were analyzed by the Glimmix procedure using the binomial option. The means were obtained by the lsmeans command, and mean comparisons were performed by the pdiff option. The age at puberty was analyzed by survival curve performed from the lifetest procedure, and the Logrank test was used to determine the difference among curves.

Results

Female calves were weaned at 88 ± 2 days, with a body weight of 84 ± 2 kg. Heifers with high ADG were 35 kg heavier than the control at 7 months (Table 2). As expected, the DMI was 60% less in heifers from control treatment in phase I. Likewise, phase II also induced a smaller ($P < 0.01$) ADG in control heifers submitted to DMI restriction (Table 3). An interaction between phases was observed for DMI ($P < 0.05$), in which HH had greater DMI than HC, CH, and CC. The CH group had greater ($P < 0.05$) DMI compared to HC and CC. The puberty rate at 19 months of age was affected ($P = 0.01$) by the treatments (Table 4; Fig. 1), in which HH treatment (84%) induced a greater ($P = 0.01$) proportion of pubertal heifers than the CC (23%). The puberty rate was similar in HC (60%) and CH (50%) treatments. In addition, the behavior of the puberty curves from HC and CH treatments were also similar throughout the whole experiment.

Table 2
Performance of Nellore heifers in phase I (from 3rd to 7th month of age).

Variables	Treatments		P-value
	High	Control	
Initial age (day)	88 ± 2	88 ± 2	0.80
Initial BW (kg)	83 ± 2	85 ± 2	0.28
BW at 7th month of age (kg)	180 ± 17	145 ± 20	< .01
ADG (kg)	0.787 ± 0.03	0.448 ± 0.03	< .01
DMI (kg/day)	4.0 ± 0.10	1.6 ± 0.03	< .01
FE	0.19 ± 0.01	0.28 ± 0.01	< .01

Abbreviations: AGD, average daily gain; DMI, dry matter intake. High, heifers submitted to high ADG with DMI *ad libitum*, AGD = 0.79 kg/day. Control, heifers submitted to restricted DMI, ADG = 0.45 kg/day; FE, feed efficiency ratio between ADG and DMI.

Table 3
Performance of Nellore heifers in phase II (from the 7th month of age to puberty or 19th month of age in non-pubertal heifers).

Variables	Treatments				P-value		
	HH	HC	CH	CC	F1	F2	F1*F2
BW at 7th month of age (kg)	183 ± 21	180 ± 15	144 ± 10	145 ± 20	< .01	.	.
ADG (kg)	0.674 ± 0.02	0.524 ± 0.02	0.717 ± 0.02	0.520 ± 0.02	0.39	< .01	0.28
DMI (kg/day)	5.5^{a} ± 0.10	2.9^{c} ± 0.05	4.7^{b} ± 0.09	3.1^{c} ± 0.06	< .01	< .01	0.05
FE	0.12^{c} ± 0.01	0.18^{a} ± 0.01	0.15^{b} ± 0.01	0.17^{ab} ± 0.01	0.02	< .01	< .01

Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II; HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II; BW, body weight; AGD, average daily gain; DMI, dry matter intake; FE, feed efficiency ratio between ADG and DMI. F1, the effect of phase I, F2, effect of phase II; F1*F2, interaction effect between F1 and F2.

Table 4
Performance of pubertal Nellore heifers during the experiment.

Variables	Treatments				P-value		
	HH	HC	CH	CC	F1	F2	F1*F2
BW at puberty (kg)	345 ± 11	325 ± 12	333 ± 17	346 ± 7	0.78	0.80	0.30
Age at puberty (months)	14.5 ± 0.4	16.0 ± 0.5	15.6 ± 0.5	17.6 ± 0.7	0.02	0.01	0.64
Total DMI (kg)	1,992 ± 75	1,440 ± 85	1,758 ± 78	1,360 ± 75	0.13	< .01	0.59
Puberty (%)	84 (11/13)	60 (6/10)	50 (6/12)	23 (3/13)			

Abbreviations: HH, heifers submitted to high ADG (DMI ad libitum) in phases I (from 3rd to 7th month of age) and II; HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II (from 7th month of age to puberty); CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II; BW, body weight; Total DMI, dry matter intake of pubertal heifers from to 3rd month of age to puberty. F1, the effect of phase I, F2, the effect of phase II; F1*F2, an interaction effect between F1 and F2.

There was an interaction among phases and age for serum leptin concentration ($P < 0.01$), which heifers from HH treatment had greater serum leptin concentration than other treatments at 13 months old. Moreover, serum leptin concentration staied elevated at the 18th month in HH compared with CH and CC (Fig. 2). However, phases and age interaction were not observed ($P = 0.68$) in serum IGF1 concentration. However, heifers submitted to high ADG during phase I had greater ($P = 0.02$) serum IGF1 concentration than control ADG at 5 and 7 months old.

There was an interaction ($P < 0.01$) among phases and age in the diameter of the largest follicle, in which heifers from HH treatment had greater follicles compared to heifers from CC in most of the months evaluated during the experiment (Fig. 3). In addition, heifers that reached puberty had greater follicles than non-pubertal heifers at 11, 13 and 14 months.

Considering the LH profile, there was no interaction ($P > 0.05$) among phases and age in any variable relative to the LH profile (Table 5). However, control heifers had greater ($P < 0.05$) frequency of LH pulses compared with heifers in high AGD in both phases at 15 and 16 months of age. In addition, pubertal heifers ($n = 10$) showed a similar LH profile compared with non-pubertal heifers ($n = 10$), regardless of treatment (Fig. 4). Despite puberty status or treatment effects on LH variables, the heifers` age was the main factor changing LH profile (Fig. 5), affecting frequency, amplitude, and maximum LH concentration. Amplitude and maximum LH concentration increased from the 7th to 9th month of age, but the frequency of LH pulses decreased at these ages. From the 9th to 17th month of age, the frequency of LH pulses increased slowly but dropped rapidly at the 18th and 19th months of age. The same pattern was observed for the diameter of the largest follicle. An example of a decrease in the frequency of LH pulses was demonstrated in Fig. 6, which showed the LH profile of a non-pubertal heifer with 179 kg and 390 kg of BW at 7 and 19 months of age, respectively.

Table 5
LH profile of Nellore heifers submitted to high (*ad libitum* intake) or control (50% restricted intake) ADG on phase I (from 3rd to 7th month of age) and phase I (from 7th to 19th month of age).

	Maximum LH concentration (ng/mL)								The amplitude of LH pulse (ng/mL)							
Age (months)	Phase I		SEM	P-Value	Phase II		SEM	P-Value	Phase I		SEM	P-Valor	Phase II		SEM	P-Value
	High	Control			High	Control			High	Control			High	Control		
7	2.66	1.97	0.70	0.11	2.39	2.25	0.48	0.81	1.87	1.20	0.68	0.52	1.63	1.40	0.52	0.70
9	4.42	3.52	0.38	0.06	4.09	3.72	0.44	0.74	3.32	2.65	0.37	0.13	3.12	2.75	0.42	0.85
11	3.20	2.53	0.40	0.22	2.68	3.30	0.41	0.27	2.21	1.70	0.39	0.43	1.75	2.38	0.40	0.33
13	3.29	3.51	0.32	0.45	2.97	3.75	0.32	0.12	2.20	2.53	0.31	0.29	1.89	2.77	0.32	0.08
15	3.93	2.86	0.32	0.15	2.86	3.37	0.32	0.54	2.72	1.85	0.31	0.15	1.87	2.25	0.31	0.09
16	3.24	2.61	0.32	0.76	2.57	2.92	0.34	0.57	2.29	1.52	0.31	0.63	1.46	1.90	0.33	0.53
17	2.97	3.24	0.33	0.41	2.52	3.56	0.33	0.09	1.90	2.09	0.33	0.39	1.34	2.47	0.33	0.07
18	2.97	3.80	0.43	0.94	3.60	3.44	0.38	0.78	1.87	2.74	0.42	0.89	2.71	2.31	0.37	0.61
19	2.05	2.99	0.57	0.84	3.44	2.23	0.74	0.30	1.08	1.82	0.55	0.25	2.26	1.13	0.71	0.12
	Mean of LH concentration (ng/mL)								Frequency of LH pulses/12 hours							
Age (months)	Phase I		SEM	P-Value	Phase II		SEM	P-Value	Phase I		SEM	P-Valor	Phase II		SEM	P-Value
	High	Control			High	Control			High	Control			High	Control		
7	1.08	1.01	0.18	0.35	1.07	1.03	0.10	0.75	2.00	1.60	0.58	0.32	2.00	1.50	0.48	0.06
9	1.11	1.06	0.11	0.75	1.01	1.15	0.10	0.34	1.11	1.30	0.32	0.35	1.09	1.38	0.29	0.69
11	1.20	0.94	0.11	0.08	1.04	1.10	0.10	0.70	1.67	1.10	0.32	0.42	1.45	1.25	0.29	0.39
13	1.31	1.21	0.10	0.53	1.23	1.29	0.11	0.69	1.80	1.82	0.31	0.91	1.55	2.10	0.30	0.28
15	1.34	1.30	0.15	0.83	1.20	1.44	0.10	0.21	1.67	2.33	0.47	0.02	1.43	2.63	0.45	0.01
16	1.01	1.15	0.15	0.44	1.06	1.10	0.10	0.84	1.60	2.22	0.48	0.02	1.67	2.25	0.46	0.01
17	1.36	1.33	0.15	0.88	1.31	1.38	0.11	0.69	2.20	2.71	0.47	0.71	3.00	2.25	0.48	0.25
18	1.29	1.27	0.16	0.91	1.22	1.34	0.12	0.54	2.33	1.83	0.51	0.42	1.00	2.80	0.48	0.02
19	1.13	1.12	0.16	0.95	1.23	1.02	0.12	0.29	0.33	0.83	0.51	0.69	0.75	0.60	0.48	0.64

There was no interaction among experimental phases and age in any variable ($P > 0.05$). The treatments were arranged in 2 x 2 factorial, whose first factor was high (H; 0.79 kg/day) or control (C; 0.45 kg/day) ADG from the 3rd to 7th month of age (growth phase I). The second factor was also high (H; 0.7 kg/day) or control (C; 0.5 kg/day) ADG from the 7th month of age to puberty (growth phase II).

At 19 months, non-pubertal heifers from HH, CH, and HC treatments were heavier than pubertal heifers in their respective treatment. Only the heifers of the CC treatment (330 ± 9 kg) were lighter than pubertal heifers (346 ± 7 kg) of the same treatment (Table 6).

Table 6
Response to puberty induction using an intravaginal progesterone device by 9 days in non-pubertal Nellore heifers at 19th month of age.

	Treatments			
	HH	HC	CH	CC
Number	2	4	6	10
Heifers with CL (%)	100	75	71	70
BW at 19 months of age (kg)	$399^{a} \pm 20$	$340^{b} \pm 14$	$401^{a} \pm 14$	$330^{b} \pm 9$

Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19th month of age); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II; CL, corpus luteum; BW, body weight;

Different letters indicate statistically significant differences among treatments ($P = 0.0019$).

At 19 months of age, we finished the experiment by inducting heifers to ovulation with the insertion of an intravaginal progesterone device, which induced a CL in around 70% of heifers (Table 6). The heifers of the HH and CH treatments presented the highest BW ($P = 0.0019$) compared to restricted heifers in phase II.

Discussion

The main objective of this study was to determine whether a high ADG in heifers early weaned accelerate puberty in Nellore heifers. In the current study, the high growth rate from the 3rd to 7th month of age did not increase the percentage of pubertal Nellore heifers at 19 months old. However, our results indicated that early weaning associated with high ADG during younger age makes heifers more efficient since puberty curves from HC and CH treatments were similar. This was not expected since HC heifers had DMI restriction for 11 months during phase II. Nonetheless, heifers presented a similar cumulative puberty proportion to CH treatment that remained for 11 months under *ad libitum* DMI. In addition, CH heifers were heavier than HC at the end of the trial, showing that CH heifers reached desired BW but did not reach puberty. In addition, HH and CH heifers showed similar BW at the end of the experiment; however, HH treatment induced close to 25% more pubertal heifers than CH treatment. Although this difference was not statistically significant, the HH treatment induced a greater percentage of puberty compared to other treatments. These data together could greatly affect farmers' costs, using less feed to induce puberty in heifers.

Our data did not corroborate the results reported by Moriel et al. (2014), who observed an accelerated puberty in Brahman × British crossbred heifers that were early weaned and submitted to a grain-based diet for 180 days. Moreover, Nellore heifers from the current trial showed smaller growth performance compared to Angus or crossbreed heifers from Gasser et al. (2006a), Cardoso et al. (2014), and Moriel et al. (2014) studies. Nellore heifers in the current study had an ADG of 0.8 kg/d and DMI of 3% of BW. In previous studies that showed the effect of metabolic imprinting before 7 months of age with the further earlier puberty, heifers had ADG greater than 1 kg/d (Gasser et al. 2006a; Cardoso et al. 2014), consuming 3% (Gasser et al. 2006a) to 3.5% (Moriel et al. 2014) of BW. Therefore, the smaller ADG observed Nellore heifers in the current trial may explain the lack of metabolic imprinting in our heifers.

Dry matter intake was around 0.5 kg/day greater in HH heifers than in CH during phase II. However, CH heifers had close to 0.1 kg/d greater ADG than HH heifers, indicating that CH heifers had a compensatory growth. Miszura et al. (2020) and Cardoso et al. (2014) reported a similar compensatory growth, which did not affect the age at puberty. However, HH heifers showed a puberty rate about 30% greater than CH heifers, but little is known about the effect of compensatory BW gain on age at puberty.

The percentage of pubertal heifers in HH treatment (84%) until 18 months of age may be considered a paradigm break in the Nellore breed. As far as we know, no studies have shown this high percentage of spontaneous *Bos indicus* pubertal heifers at this age. Notably, there was a great genetic effect in our experiment as all heifers were daughters of the same sire that may be considered precocious for the breed standards [the best 1% for scrotal circumference in the Aliança (2019) catalog. Previous studies have indicated that genetics was more important than nutrition for puberty in *Bos indicus* heifers (Ferraz Junior et al. 2018). The current study observed that high ADG associated with early weaning in heifers with genetics favorable to sexual precocity was a tool to induce a greater percentage of puberty in heifers before their first breeding season.

The high ADG induced greater serum leptin concentration in HH from the 13th to 18th month of age, indicating that HH treatment could change the body composition of heifers, which increases fat percentage in heifers. Probably, greater serum leptin concentration was determinant in inducing a greater percentage of puberty in this treatment. However, Ferraz Junior et al. (2018) did not observe differences in serum leptin concentration in precocious and non-precocious Nellore heifers. Previous studies have shown that leptin infusion in the brain in fasting mice stimulated GnRH and LH secretion (Watanobe 2002). However, experiments with primates and rodents did not confirm this direct action of leptin on GnRH-secreting neurons (Roa et al. 2010). Leptin can inhibit NPY expression (Gamba et al. 2006), modulate the expression of kisspeptin receptors (Stephens et al. 2015), and then hasten puberty. However, although chronic administration of recombinant leptin increased leptin concentration, it did not increase the frequency of LH pulses nor anticipated puberty in beef heifers (Maciel et al. 2004; Carvalho et al. 2013). Therefore, it is assumed that leptin is not responsible for puberty triggers, but acts as a permissive signal for puberty (Maciel et al. 2004; Barb et al. 2004).

The high ADG from the 3rd to 7th month of age increased serum IGF1 concentration at 5 and 7 months. A greater amount of grain in the diet increases insulin concentration, stimulating IGF1 production through an increase in the hepatic expression of growth hormone receptors (GHR-1A) (Butler et al. 2003). However, a high ADG at phase II was unable to increase serum IGF1 concentration compared with control heifers, likely to the smaller difference in ADG at phase II compared with I (0.170 and 0.340 kg/day, respectively). Heifers submitted to *ad libitum* intake (HH, CH, and HC treatments) had a serum IGF1 peak at 12 months old, just before heifers reaching puberty. Notably, a serum IGF1 increase has been associated with puberty onset (Cooke et al. 2013; Johnston et al. 2014; Ferraz Junior et al. 2018), but greater serum IGF1 was not able to induce all heifers to puberty. Therefore, serum IGF1, similar to serum leptin, is also not responsible for puberty triggers but acts as a permissive signal for puberty. Taking together leptin and IGF1 data, greater serum leptin concentration was more associated with greater BW. On the other hand, greater serum IGF1 was more associated with increasing DMI.

Different BW induced small effects on LH profile, likely due to the smaller difference in ADG among treatments. Furthermore, the LH profile was probably more refractory to nutritional treatments because of the great inhibition of neurons GnRH producers in *Bos indicus* than in *Bos taurus* heifers. In Angus heifers, it was demonstrated that a decline in negative feedback exerted by estrogen in GnRH was necessary to increase the frequency of LH pulses during the prepubertal phase (Day and Anderson 1998). In the current trial, the main factor affecting LH profile was age, whose frequency of LH pulses slowly increased from the 9th to 17th month of age, but it occurred in both pubertal and non-pubertal heifers. This result disagrees with studies with Angus heifers that showed an increase in the frequency of LH pulse before puberty (Day and Anderson 1998; Gasser et al. 2006d), which normally is used as a good predictor of puberty. However, Rodrigues et al. (2002) reported that the frequency of LH pulse increased before puberty in *Bos taurus* heifers but not in *Bos indicus* heifers. Little is known about the LH profile in Nellore heifers before reaching puberty. We suggest that the first ovulation in puberty Nellore heifers occurred, beyond the LH frequency increase, due to follicular mechanisms that allowed the dominant follicle to get ovulation capacity from an increase of LH receptor and then ovulated with 2 to 2.5 LH pulses/12 hours. This idea was corroborated by the greater largest follicle diameter from the 11th to 14th month of age in pubertal heifers despite a similar LH profile in puberty heifers. However, non-pubertal heifers also showed a similar increased frequency of LH pulse, which was

corroborated with the similar diameter of the largest follicle since the 15th month of age. However, the current study was not able to explain why non-puberty heifers had a similar increase in the frequency of LH pulse and the largest follicle diameter, as well as leptin and IGF1 concentration.

From the 17th to 19th month of age, there was a drop in the frequency of LH pulse and the largest follicle diameter, and none of the heifers reached puberty at this age, even with all heifers having greater BW and an ascendant nutritional plan. In June, the winter solstice occurs in the southern hemisphere. Thus, we speculate that heifers had a photoperiod effect, in which short days could block puberty onset. In the same location, no heifers reached puberty from May to August for 2 consecutive years (Ferraz Junior et al. 2018). However, photoperiod in heifers is poorly studied.

The induction of puberty with progesterone has grown in Brazilian livestock (Claro Júnior et al. 2010; Rodrigues et al. 2014). At 24 months of age and about 330 kg of body weight, it is possible to induce puberty in about 90% of heifers (Rodrigues et al. 2014). In the present study, the insertion of a source of progesterone was able to induce ovulation in 70% of non-pubertal Nellore heifers at 19 months of age, regardless of body weight that ranged from 330 to 401 kg. Progesterone is effective in inducing puberty in Charolais and Hereford heifers from 12.5 months old or greater Hall et al. (1997). The mechanism by which progesterone induces puberty is not yet well known. Still, it is known that kisspeptin neurons are sensitive to progesterone, and progesterone receptors are needed to induce a normal LH peak via kisspeptin Hall et al. (1997).

In conclusion, high ADG was associated with earlier puberty, mainly with high ADG at younger age. The programmed fed applied in the current study increased serum leptin in HH heifers after receving a grain-based diet. On the other hand, serum IGF1 was more affected by changes in DMI. In addition, this study showed that LH profile was mainly affected by age.

Declarations

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Competing Interests

The authors declare that they have no conflict of interest

Author Contributions

M.V.C. Ferraz: Data curation, Investigation, Writing - original draft. E.M. Ferreira: Investigation, Funding acquisition. M.H. Santos: Investigation. G.B. Oliveira: Investigation. J.P.R. Barroso: Investigation. D.M. Polizel: Investigation. G.P. Nogueira: Conceptualization, Methodology; V.N. Gouvea: Conceptualization, Methodology. P.H.V. Carvalho, Writing - review & editing; J.S. Biava Investigation, Writing - original draft. E.M. Ferreira: Writing - original draft. A.V. Pires Methodology, Conceptualization, Funding acquisition, Supervision. All authors read and approved the manuscript.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval

The Animal Care and Use Committee from the University of São Paulo approved all procedures with animals.

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Figures

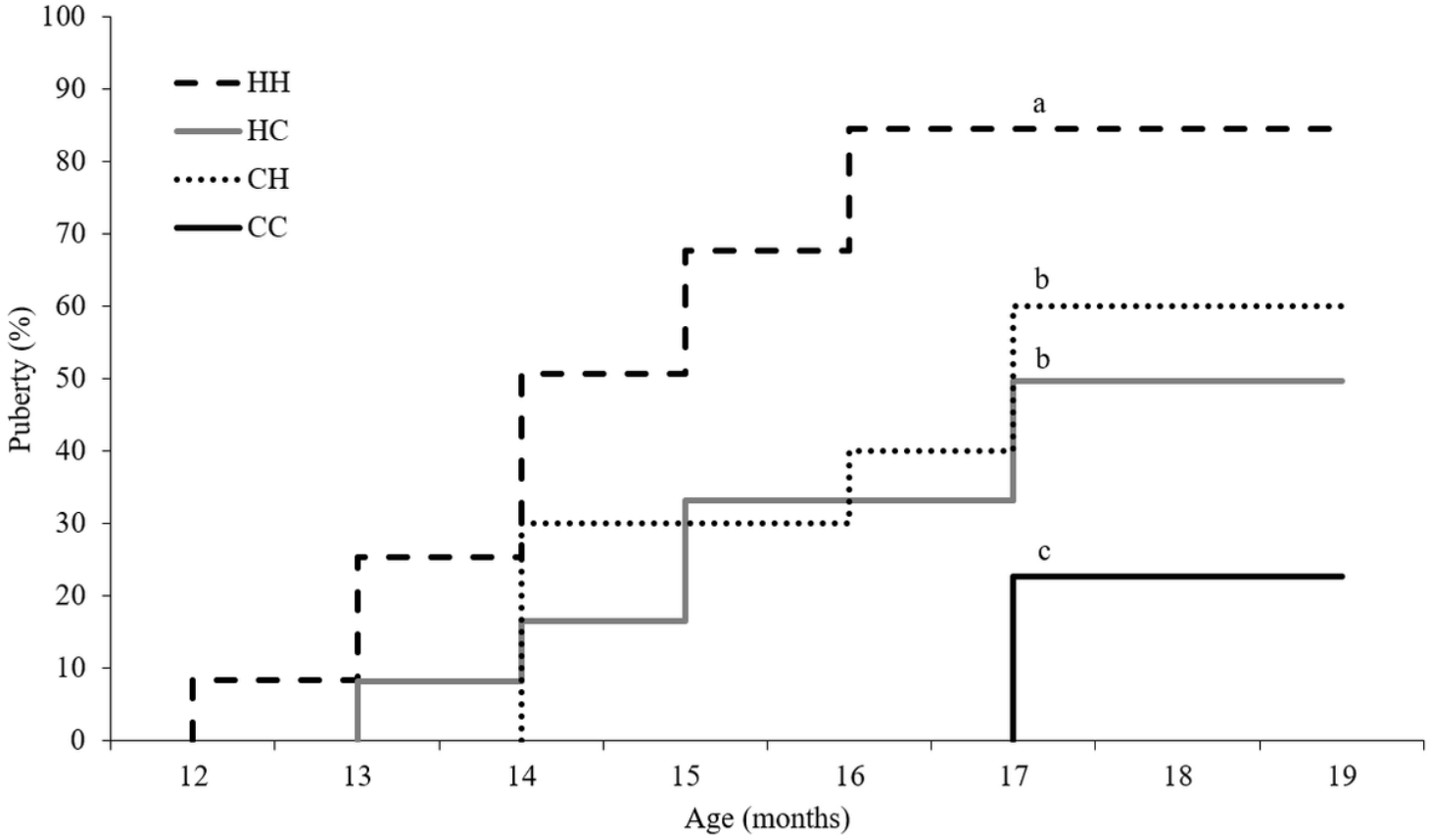

Figure 1

The cumulative proportion (%) of Nellore heifers attained puberty in each treatment until the 19th month. $^{a-b}$ Lines with different lattes were statically different (P = 0.0145). Heifers aged 12 months in December. Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19th month of age); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II;

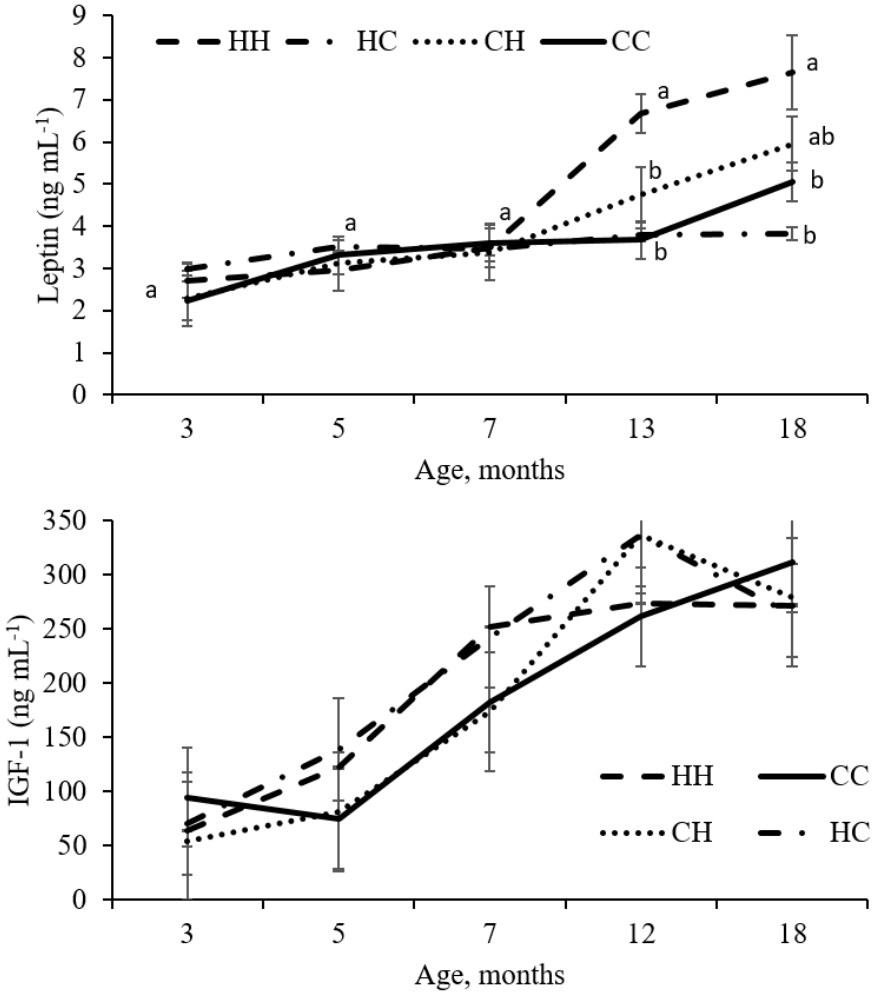

Figure 2

The serum concentration of IGF1 and leptin according to treatments at 3, 5, 7, 13, and 18 months of age. There was an interaction of leptin concentration among phases and age (P = 0.0026), with was demonstrated by different letters. There was no interaction in IGF1 concentration between treatments and age (P = 0.6829), but heifers submitted to high ADG during phase I had higher (P = 0.0220) IGF1 concentration than control at 5 and 7 months of age. Abbreviations: HH, heifers submitted to high ADG (50% DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th to 19th month of age); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II;

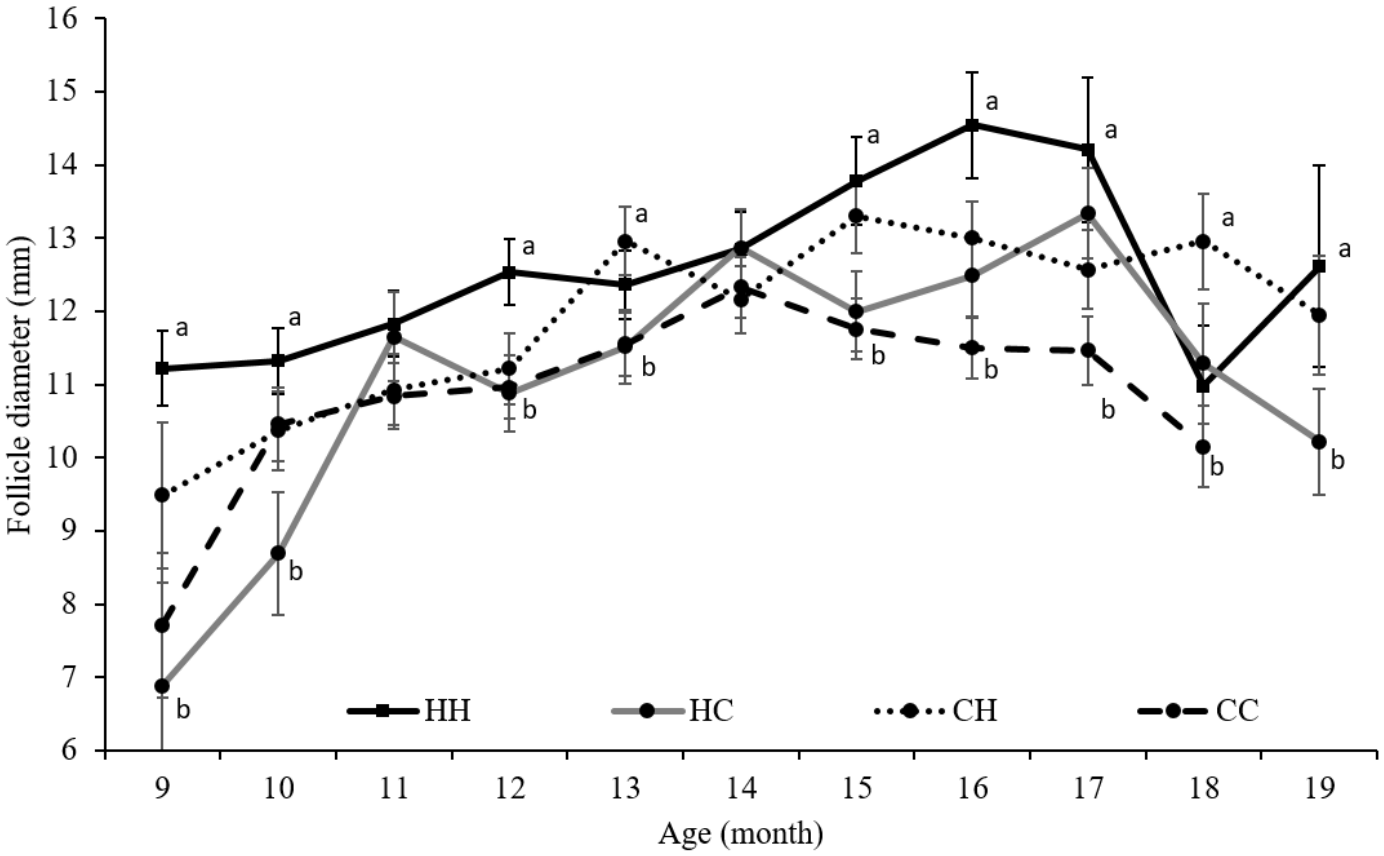

Figure 3

Effect of treatment on the diameter of the largest follicle in prepubertal Nellore heifers. Ovaries of heifers were scanned every 2 days for 15 days once a month to determine the maximum diameter of the follicle. There was an interaction between factors and age (P = 0.0221). $^{a-b}$ Lines with deferment lattes were statically different (P < 0.05). Abbreviations: HH, heifers submitted to high ADG (DMI *ad libitum*) in phases I (from 3rd to 7th month of age) and II (from 7th month of age to puberty); HC, high ADG in phase I and control (50% DMI restricted) ADG in phase II; CH, control ADG in phase I and high ADG in phase II; CC, Control ADG in the phases I and II;

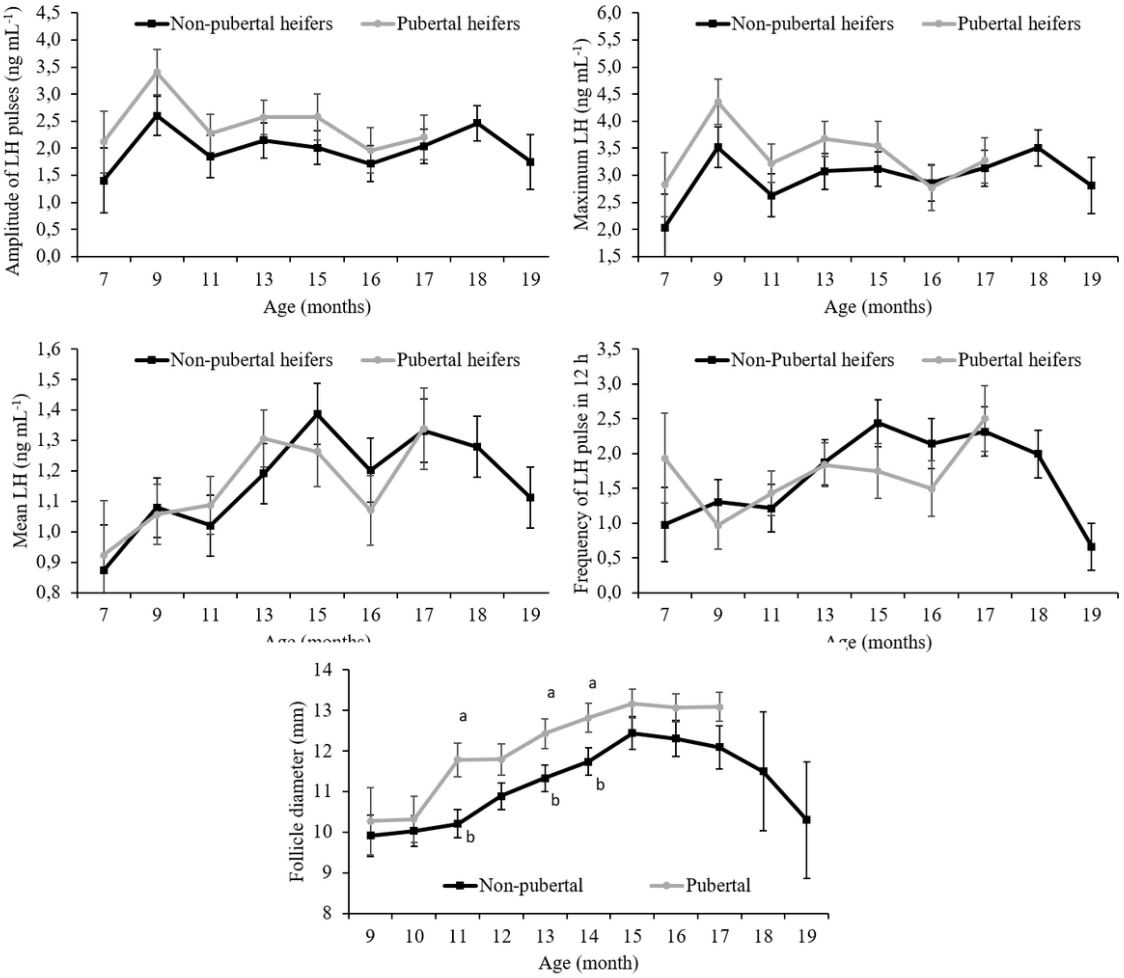

Figure 4

The effect of puberty status until the end of the experiment on LH profile and the largest follicle diameter in Nellore heifers. Blood samples were taken before puberty, and after the first ovulation, heifers were removed from the experiment. Ten heifers that were selected for LH blood collection reached puberty. There was no effect ($P > 0.05$) of puberty status and no interactions between puberty status and age in any LH profile variable. However, at 11, 13, and 15 months, pubertal heifers had larger follicles ($P < 0.05$) than non-pubertal heifers.

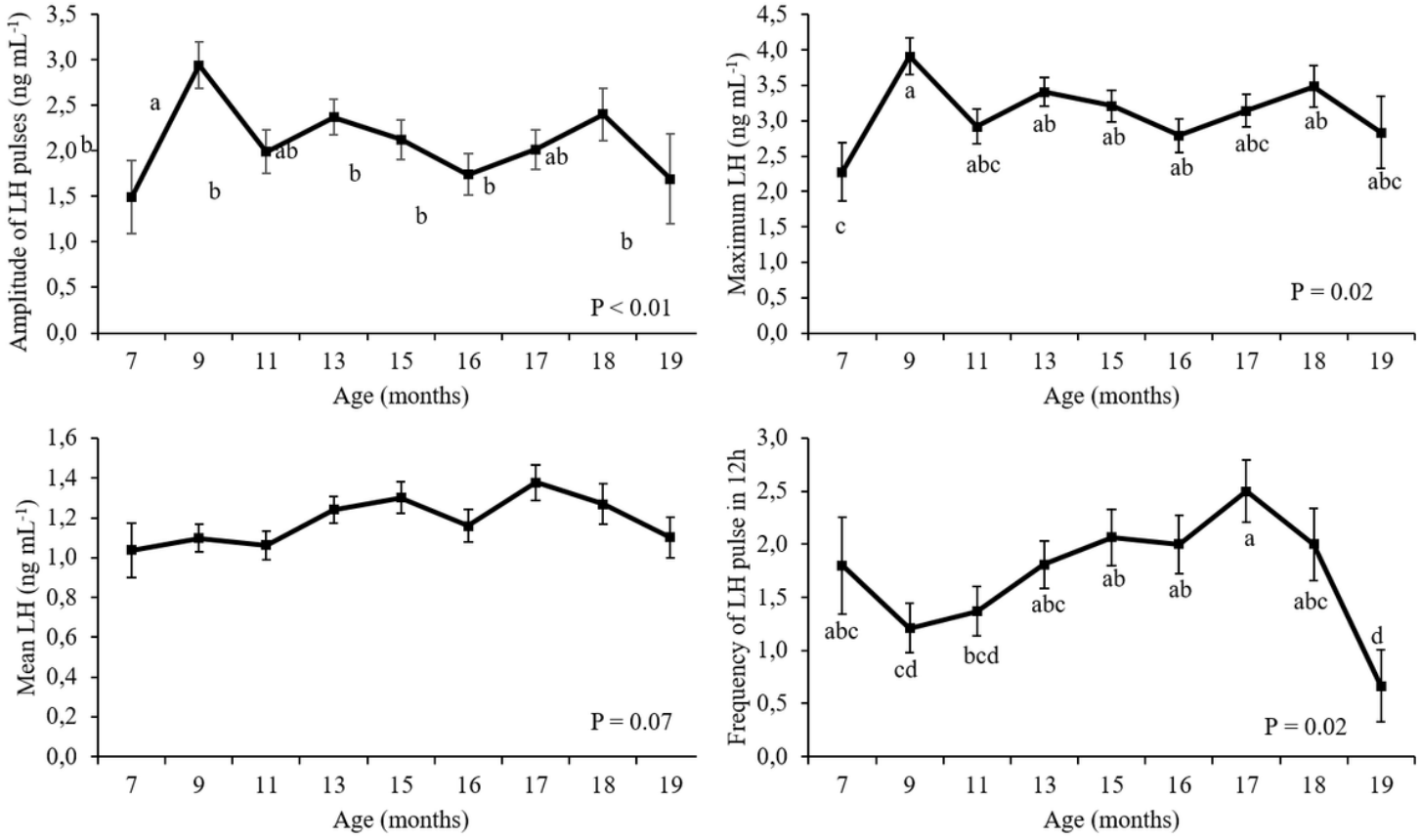

Figure 5

Effect of age in LH profile in Nellore heifers. [a – b] Lines with deferment lattes were statically different (P < 0.05).

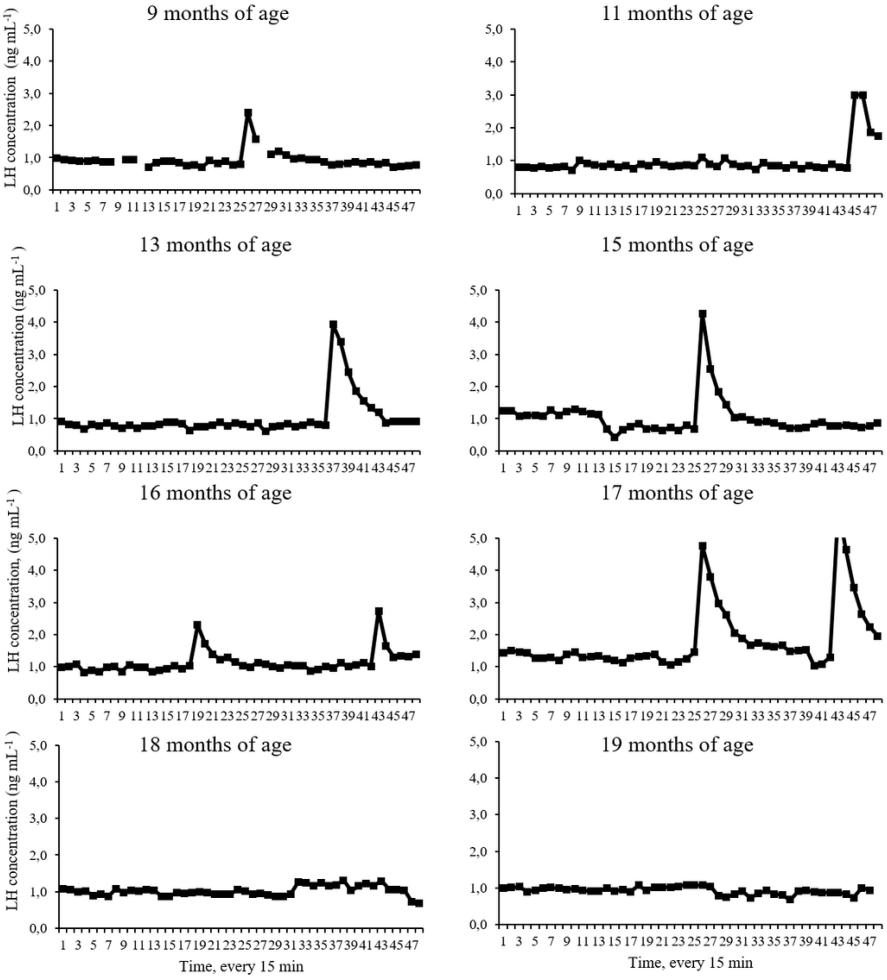

Figure 6

LH profile of a non-pubertal Nellore heifer with a body weight of 179 kg and 390 kg at the 7th and 19th month of age, respectively. Serum samples were collected every 15-minute intervals for 12 hours—heifer aged 12 months in December.