Body and testicular traits, libido testing and semen analysis of young males from the Blanco Orejinegro (*Bos taurus*) herd of University of Antioquia, throughout puberty onset.

German Urrea[1], MV; Sandra L. Cordoba[1], MV; Jorge A. Neira[2,3], MVZ, MS;
Juan Maldonado-Estrada[1,4], MVZ, MS.

[1]Grupo de Teriogenología, Facultad de Medicina Veterinaria y de Zootecnia, y Programa de Biogénesis, Facultad de Medicina, Universidad de Antioquia, AA 1226, Medellín, Colombia.
[2]Centro de Investigaciones El Nus, Corpoica. AA 100, Rionegro, Antioquia.
Present address: [3]Ecole Vétérinaire de Nantes, Nantes, France; [4]Unité 131 INSERM, Equipe Cytokines et Immunorégulation Maternelle, Hôpital A. Béclère. 157, Av. de la Porte de Trivaux. 92141 Clamart, France.

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Summary

Fifteen young males (mixed ages) from the BON herd of University of Antioquia were selected and followed weekly from August to December 1998 (Comprising a range from 9,9 to 28,1 months, from 19 time points of data collection) in order to measure and evaluate: 1) Body and Testicular traits (n = 322); 2) Libido testing (LibT, n = 19); 3) Response to rectal palpation of seminal vesicles reflex (SVR, n = 244); and 4) Analysis of semen collected by artificial vagina (n = 42). Data of physical traits were categorized monthly from 10 to 27 months (n = 17 groups); and data of semen were categorized into three groups (16,1 to 21; 21,1 to 24; and 24,1 to 27 months). Simple regression analysis was performed between each trait (dependent variable) and age (independent variable). Multivariate analysis of variance was used to establish differences between groups and monthly reference values for each trait. Physical traits proportionally increased ($p<0,01$) with age: Body Weight ($R^2=0,796$) and Scrotal Circumference ($R^2 = 0,672$), had the highest correlation coefficients, whereas Body Length ($R^2 = 0,454$), Withers height ($R^2 = 0,506$), Testis Diameter ($R^2 = 0,415$), and Testis Length ($R^2 = 0,458$), had a mild correlation. Higher scores for LibT (more than 5 mating attempts/5 minutes) were always observed. Individual responses to SVR (secretion of pre-seminal fluid, protrusion of penis and complete erection) proportionally increased with age ($R^2 = 0,539$; $p<0,01$). The youngest age at semen collection was 16,1 months ($1,63 \times 10^8$ sperm/cc, with >60% gross and individual progressive motility); however, no correlation was found with age, neither for volume (3,6 ml), nor for pH (6,86) or semen concentration. These results suggest the onset of puberty occurs between 13 and 14 months for this BON herd and confirm previous observations on the high libido of BON males. Reference values are reported for both testicular and body traits. Breeding Soundness Examination rather than merely semen concentration must be considered in order to define the onset of puberty in bovine cattle.

Key words: *Bovine, Criollo Cattle, Puberty, Semen, Tropical Environment, Young Bulls.*

Introduction

Blanco Orejinegro (BON) cattle (*Bos taurus*) is one of the greatest herd of tropical-adapted Colombian Criollo Cattle (15). Bred in several pure and cross-bred nuclei at the coffee plantations areas of Colombia (27), BON is a genetic resource adapted to fit the conditions of Colombian tropic, showing good fitness to adverse environments, including bit and fly resistance, haemoparasite resistance, and grassing on poor soil conditions (2, 3, 34); however, this "resistance" remain to be characterized by both

clinical and molecular immunogenetic studies (27, 28). Cross-breeding of BON with both Holstein in which F1 females had good milking performance and great reduction of mastitis and placenta retention incidences (University of Antioquia and University Nacional at Medellín, unpublished results) and Zebu (*B. indicus*) cattle (2, 34), resulted in increased resistance and high performance for several economically important traits.

Because of BON cattle is enlisted as an endangered genetic resource (15), several efforts have been focused on the study of its reproductive biology (30, 32) with the aim of contributing to its preservation and propagation. In 1995, a multicentric collaborative study was started in order to characterize the genetic resistance and fitness of BON cattle (15, 27, 28). The results of a recent work carried out with yearling males from the BON herd of "Centro de Investigaciones El Nus" Corpoica (Colombia), suggested puberty onset around 14 months of age for this herd (13). The present study was designed to evaluate by Breeding Soundness Evaluation, BSE (6-9) the onset and establishment of puberty in young males from the Universidad de Antioquia BON herd, which together with the Corpoica ones, represents the greatest herds of BON cattle in Colombia.

Table 1. Age and weight characteristics of young males from the University of Antioquia BON herd, included in the study.

Male identification[a]	Age (months)		Weight (kg)	
	Initial	Final	Initial	Final
23-7	9,93	12,47	232,5	245
21-7	10,36	12,89	273	286
17-7	11,28	15,66	214	256
15-7	12,53	16,91	234,5	258
11-7	16,12	20,49	290	328
5-7	18,09	20,39	267	321
1-7	18,75	23,13	279	337
37-6	20,56	24,93	318	374
29-6	22,2	26,58	317	391
27-6	22,27	26,64	354	388
25,6	22,47	26,84	368	412
23-6	22,57	26,94	318	367
21-6	22,63	24,93	417	452
17-6	22,89	25,2	361	420
11-6	25,79	28,09	384	448

[a]Young males are chronologically ordered from youngest (Born in 1997) to oldest (Born in 1996)

Material and Methods

This study was conducted from August to December 1998 at "El Progreso" farm property of University of Antioquia, located at 40 km north of Medellín, in a humid subtropical forest (Bosque húmedo subtropical, bhsT) with 1300 m.o.s.l, 24°C average temperature, 2000 mm annual rainfall, and 85% relative humidity (1). Males were fed *ad libitum* with *Brachiaria decumbens*, and Guinea *(Hypharrenya ruffa*) grass and supplemented with a local-prepared mineralized salt.

Inclusion criteria for the study. All young males bred in the farm having normal health condition were selected (n = 15) and were weekly evaluated from August to December 1998. Data recorded at each date of evaluation comprised a range of age between 9,9 and 28 months and a range of weight from 232 to 448 kg (Table 1).

Physical traits: 1) body traits were measured under restrained conditions as follows: Body weight (BD), was recorded by using an electronic balance (Tru-Test, New Zealand; error = 5 kg); withers height (WH), was measured from the bottom (ground) to the top of the scapula, with a fixed wood rule; Body length (BL), was measured with a linear meter along the dorsal axis from wither to the first *coccigeum vertebrae*. 2) testicular traits were evaluated according to the method proposed by Barth (1996): Scrotal circumference (SC), was measured with a linear meter, Testis diameter (TD) and length (TL) were both measured in the right testis by using a standard calibrator (Vernier Caliper, Russia).

Libido testing (LibT) and serving capacity. Young males were exposed in groups of 5 animals to restrained PGF_2-induced estrous cows in order to evaluate libido and serving capacity, which was categorized according to the system proposed by Barth (Which propose the observation of mating attempts during 20 minutes) as follows (6): Very high (7 or more mating attempts), High (4 to 6 mating attempts), Medium (2 to 3 mating attempts), and Low (0 to 1 mating attempts).

Semen analysis. All males that yield semen samples always exhibited very high scores for LibT.

Semen was collected between 7:00 to 10:00 hours by the artificial vagina method, and was immediately evaluated as previously reported (6): 1) Macroscopic evaluation: volume was measured by using of graded tubes and direct observation immediately after the ejaculate was recovered; color, was classified as white, yellowish, or brown; and aspect, was graded as creamy (high concentration), milky (mild concentration) or aqueous (low concentration); and 2) Microscopic evaluations (carried out within the 5 minutes after the ejaculate was recovered): progressive motility -gross and individual- was graded as very good (80 to 100%), good (60 to 80%), regular (30 to 60%) or poor (< 30%); and semen concentration was evaluated in Neubauer chamber and defined as sperm/cc of ejaculate. All macro and microscopic evaluations were performed by trained technicians.

Seminal vesicle palpation reflex. The reflex to seminal vesicle (SVR) palpation, (6) was arbitrary classified according to intensity of contractions, as strong, middle, or slight. In addition, presence of pre-seminal fluid, protrusion of penis or complete erection, were found to be proportionally related to the intensity of response to rectal massage. All those evaluations were performed by the same investigator.

Statistical analysis. Data were analyzed by simple regression, in which age was included as independent variable and each trait was analyzed as dependent variable; therefore, a multivariate analysis of variance was used to define reference values for each group of age. The Least Square Differences (LSD) were used to define significances between groups of age. These analysis where performed by using Statgraphic Plus (At University of Antioquia, Medellín, COlombia) and confirmed in StatView (At Unité 131 INSERM, Clamart, France) software statistics.

1. In order to evaluate morphological traits, age of young males recorded at each time point of evaluation was categorized monthly from 10 to 27 months. For multivariate analysis, age was included as independent variable, each trait as independent variable, and categories of age (n = 17) as factor.
2. In order to evaluate semen analysis, age at successful collections was classified as: Group 1 (16,1 to 21 months), Group 2 (21,1 to 24 months), and Group 3 (24,1 to 27 months).

Results

1. Body and testicular traits

A strong relationship was found between age and each trait (Figures 1 and 2); the highest correlation coefficients were found for BW ($R^2 = 0,796$, $p < 0,01$, Figure 1A) and SC ($R^2 = 0,672$, $p < 0,01$, Figure 2C). In addition, a mild significant correlation was found for BL ($R^2 = 0,454$, $p < 0,01$, Figure 1B) and WH ($R^2 = 0,506$, $p < 0,01$, Figure 1C), as well as for TD ($R^2 = 0,415$, $p < 0,01$, Figure 2A) and TL ($R^2 = 0,458$, $p < 0,01$, Figure 2B).

Values for BW range from 251 ± 7 to 433 ± 5 kg, at the lowest (10 to 11 months) and highest (26,1 to 27 months) categories of age, respectively (Table 2), representing a growing rate of 10,7 kg/month for the interval. A three modal curve was found for BW since starting at 10 months, followed by a significant decrease at 12 months, then a significant increase between 13 and 15 months, and between 20 and 23 months (Figure 3). Data from BW fitted a polynomial curve from which interpolations could be made for analysis of individual cases. The general equation for the model was defined as:

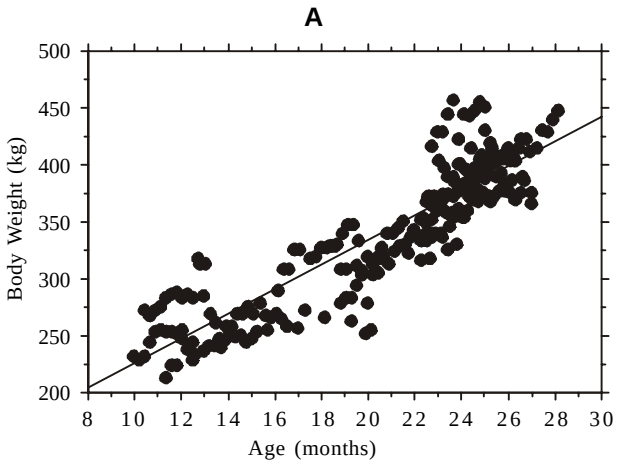
BW = 118,214 + 10,785 x Age (in months); $R^2 = 0,796$; $p < 0,01$ (Figure 1A).

Body length range from 118 ± 3 to 131 ± 1 cm, at the lowest and highest categories of age, respectively (Table 2), representing a growing rate of 0,76 cm/month for the interval. The general equation for the model was defined as:

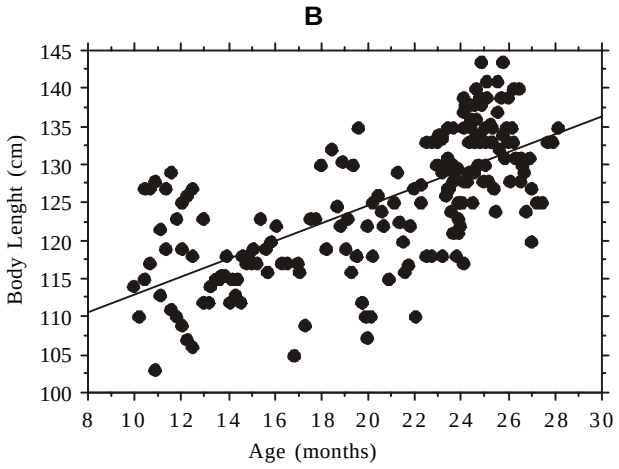
BL = 101,102 + 1,171 x Age (in months); $R^2 = 0,454$; $p < 0,01$ (Figure 1B).

Withers height (WH) range from 112 ± 2 to 121 ± 1 cm, at the lowest and highest categories of age, respectively (Table 2), representing a growing rate of 0,52 cm/month for the interval. The growth curve for WH show two intervals of faster growth from 14 to 16 and from 19 to 22 months of age (Figure 4). The polynomial transformation of the data show a growth increase since 13 months of age (Figure 4). The general equation for the model was defined as:

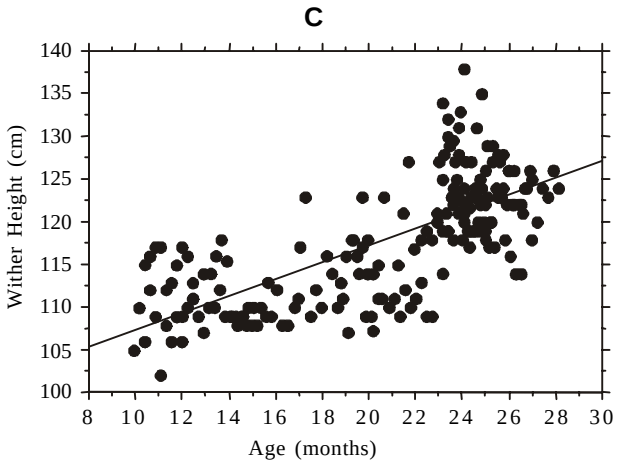
WH = 97,551 + 0,983 x Age (in months); $R^2 = 0,506$; $p < 0,01$ (Figure 1C).



Y= 118,214 + 10,785 * x; R^2 = ,796

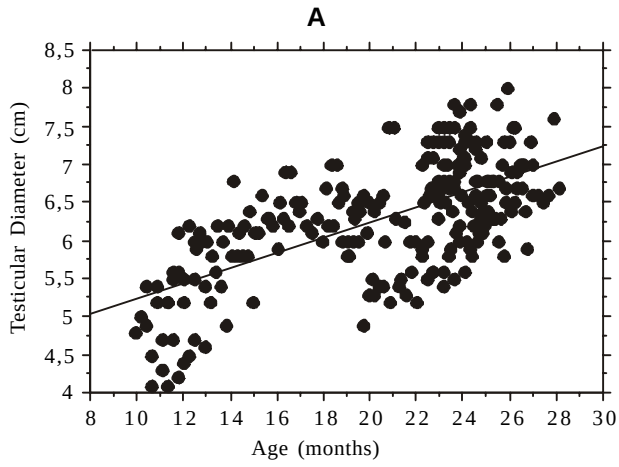


Y= 101,112 + 1,171 * x; R^2 = ,454

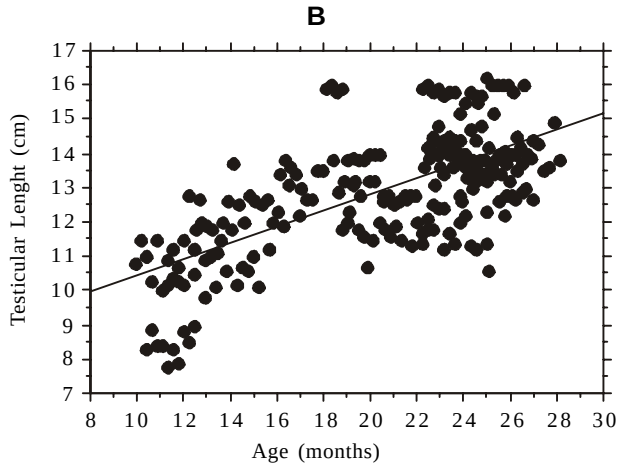


Y= 97,551 + ,983 * x; R^2 = ,506

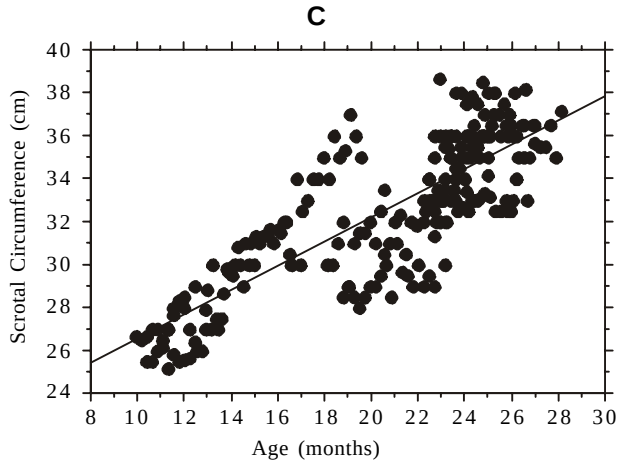
Figure 1. Simple regression for body weight (A), length (B) and withers height (C) in young males of the University of Antioquia BON herd throughout puberty onset. The equation for each model is presented at the bottom of each figure.



Y= 4,244 + ,1 * x; R^2 = ,415



Y= 8,064 + ,237 * x; R^2 = ,458



Y= 20,892 + ,563 * x; R^2 = ,672

Figure 2. Simple regression for testis diameter (A), testicular length (B) and scrotal circumference (C) in young males of the University of Antioquia BON herd throughout puberty onset. The equation for each model is presented at the bottom of each figure.

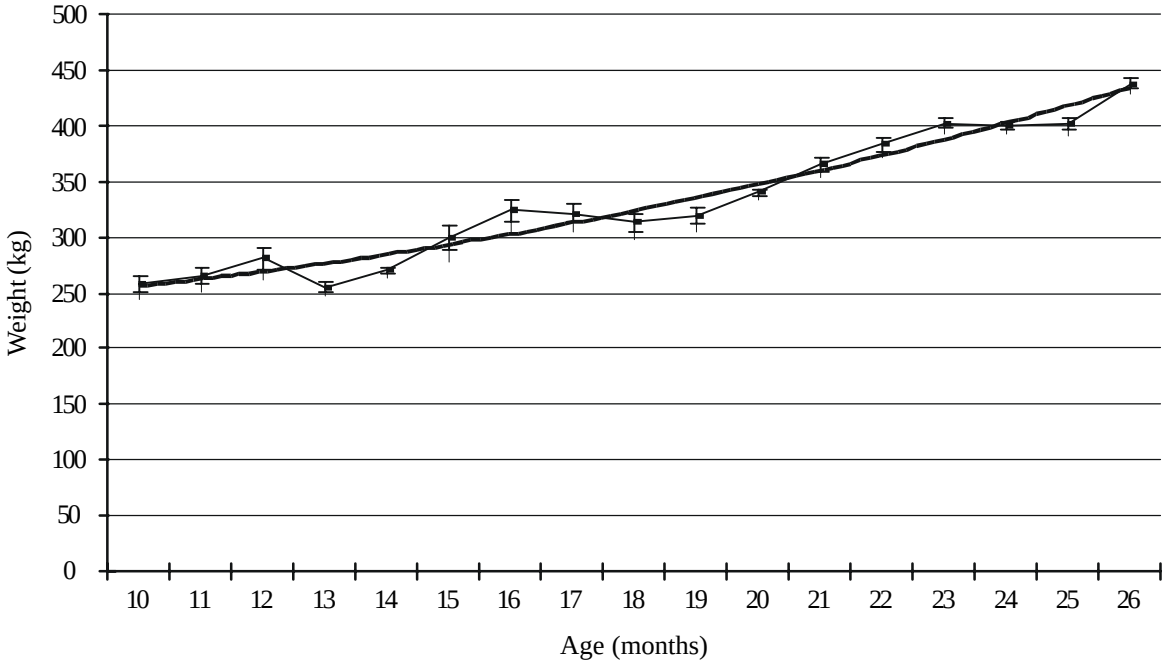


Figure 3. Growth curve of body weight in young males of the University of Antioquia BON herd, throughout puberty onset*

Polynomial transformation of data is presented as a continuous line.
*Values are presented as means (± s.e.).

Testis diameter (TD) range from 4,9 ± 0,16 to 6,8 ± 0,2 cm, at the lowest and highest groups of age, respectively (Table 3), representing a growing rate of 1,1 mm/month for the interval. The growth curve for TD show a progressive increase from 11 to 18 months when it reached the adult value observed at 27 months (Figure 5); in addition, the polynomial curve of the data show a plateau at 21 months (figure 5). The general equation for the model was defined as:

TD = 4,244 + 0,1 x Age (in months); R^2 = 0,415; p < 0,01 (Figure 2A).

Testis length (TL) range from 10,1 ± 0,5 to 14 ± 0,3 cm, at the lowest and highest groups of age, respectively (Table 3), representing a growing rate of 2,2 mm/month for the interval. The growth curve for TL as well as the polynomial curve of data, show the same pattern of TD, and adult values were also reached at 18 months (Figure 5, Table 3). The general equation for the model was defined as:

TL = 8,064 + 0,237 x Age (in months); R^2 = 0,458; p < 0,01 (Figure 2B).

Scrotal circumference (SC) range from 26,4 ± 0,2 to 35,9 ± 0,4 cm, at the lowest and highest groups of age, respectively (Table 3), representing a growing rate of 5,5 mm/month for the interval. The growth curve for SC show a progressive increase from 12 to 17 months, then significantly decrease until 21 months, with a final increase from 21 to 24 months when it reaches a plateau; the polynomial curve of the data show a progressive increase from 10 to 27 months (figure 5). The general equation for the model was:

SC = 20,892 + 0,563 x Age (in months); R^2 = 0,672; p < 0.01 (Figure 2C).

2. Libido testing and service capacity

Since the first time when BON young males were exposed to restrained PGF_2-induced estrous cows, all males exhibited a very good score (more than seven mating attempts/5 minutes), and this behavior remained throughout the study. Slight variations were observed when males were evaluated under high environmental temperatures, the presence of foreign personnel, mating competition, or due to variation in the intensity of estrous signs exhibited by the restrained cow.

3. Seminal Vesicle Reflex (SVR).

Most of the males show SVR with predominating strong (66.8%) and middle (32.3%) responses through the period of evaluation (Figure 6). In addition, response to massage of seminal vesicle by rectal palpation was evaluated in terms of pre-seminal fluid secretion, penis protrusion or complete erection, and all these signs proportionally increase ($p < 0,01$) with age (Table 4).

4. Semen analysis

The youngest age at which semen was successfully collected by artificial vagina in this work was at 16,1 months of age in male 11-7, with a volume of 3,2 ml, pH 6, having 1,63 x 10^8 sperm/cc. Most of the males produced semen having a white (50%) or yellowish color (47,6%) with a predominating aqueous (95,2%) aspect. Neither volume of semen nor pH (which remained near to 6,8 for all groups of age) significantly differed between groups of age. Similarly, semen concentration did not significantly differ between groups (Table 5). Finally, more than 60% of samples showed "very good" mass and individual progressive motility, whereas only few samples showed a lower grade of classification for this trait (Table 5). No significant relationships were found neither between age and volume ($Y = 1,68 + 0,88$ x age (in months); $R^2 = 0,027$; $p > 0,05$), nor between age and pH ($Y = 6,778 + 0,04$ x age (in months); $R^2 = 0,001$; $p > 0,05$) or age and concentration ($Y = 184,568 – 2,944$ x age (in months); $R^2 = 0,58$; $p > 0,05$).

Discussion

BON cattle is a genetic resource enlisted as endangered (15) regardless of several efforts for maintaining a breeding herd, made up by governmental organisms such as University of Antioquia and Corpoica. The conservation and propagation of BON cattle is a priority program in both of these institutions, regarding its biological value as a domestic animal genetic resource (15, 27, 28). BON exhibits a natural resistance (in way of characterization) against several of the common bacterial and parasitic pathogens of bovine, which qualify it as a prioritary candidate for genetic resistance studies. As part of several efforts focused on the study of BON cattle reproductive biology (15, 28, 30, 32), a collaborative study was started in 1997 between our group at University of Antioquia and Corpoica at C.I. El Nus, Antioquia, Colombia, in order to study and characterize puberty onset in young males of the Corpoica (13) and University of Antioquia BON herds.

Slight variations could be observed between several nuclei of BON cattle, regarding average weight and

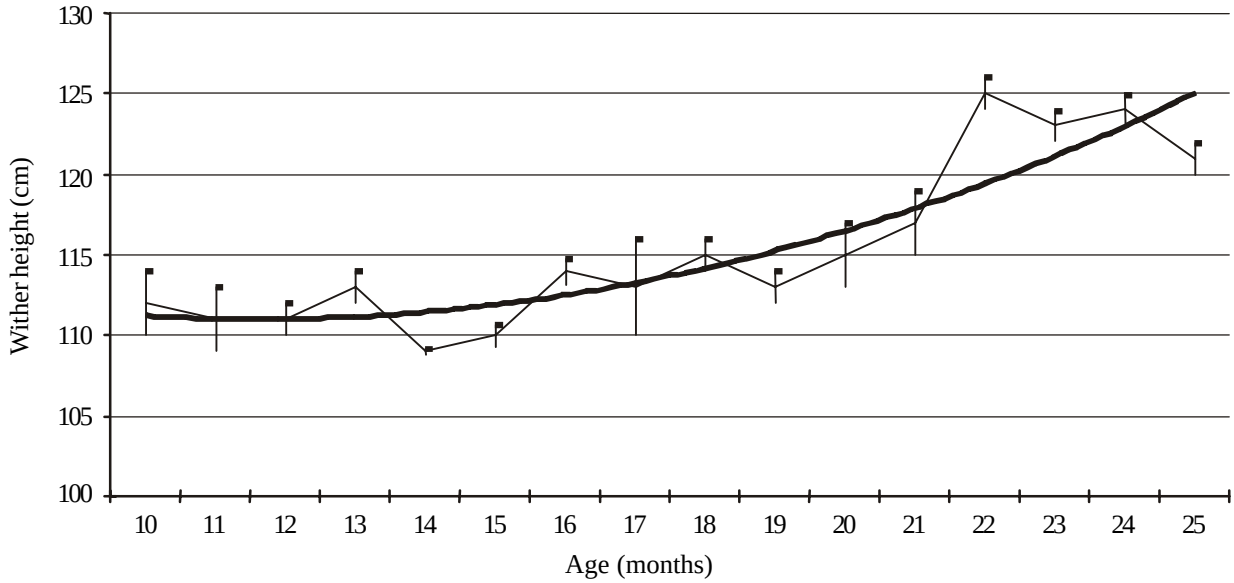


Figure 4. Growth curve for wither height in young males from University of Antioquia BON herd throughout puberty onset

Polynomial transformation of data is presented as a continuous line.
Values are presented as means (± s.e.).

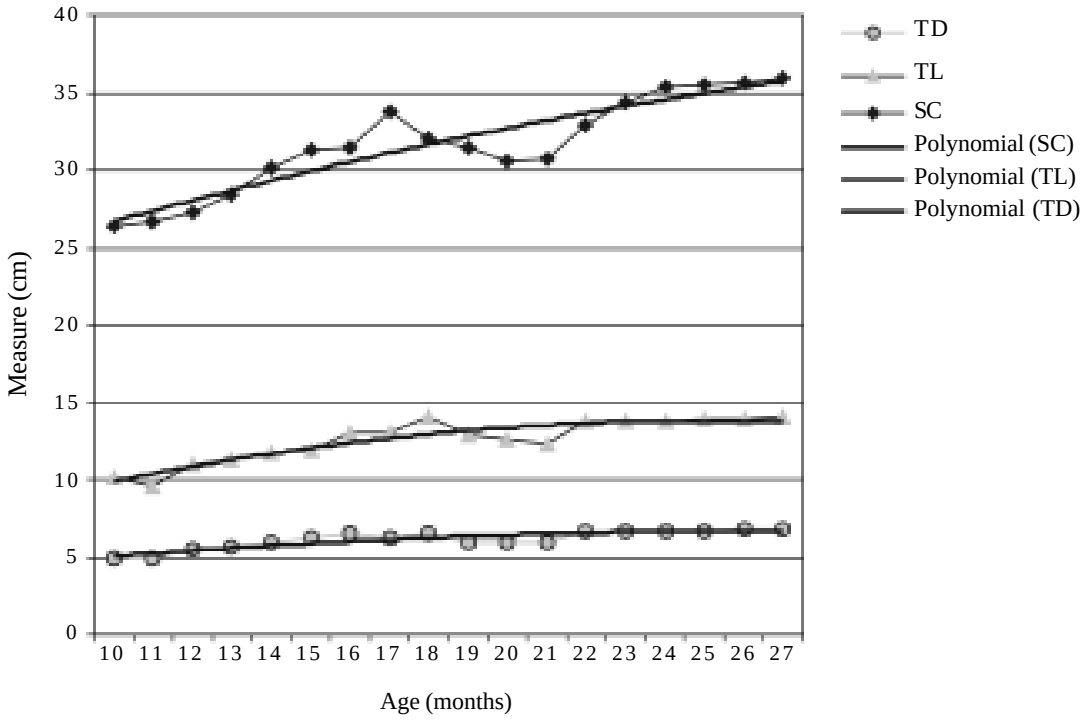


Figure 5. Curves for testicular traits in young males of the University of Antioquia BON herd throughout puberty onset

Polynomial transformation of data are presented as continuous lines.
Values are presented as means.

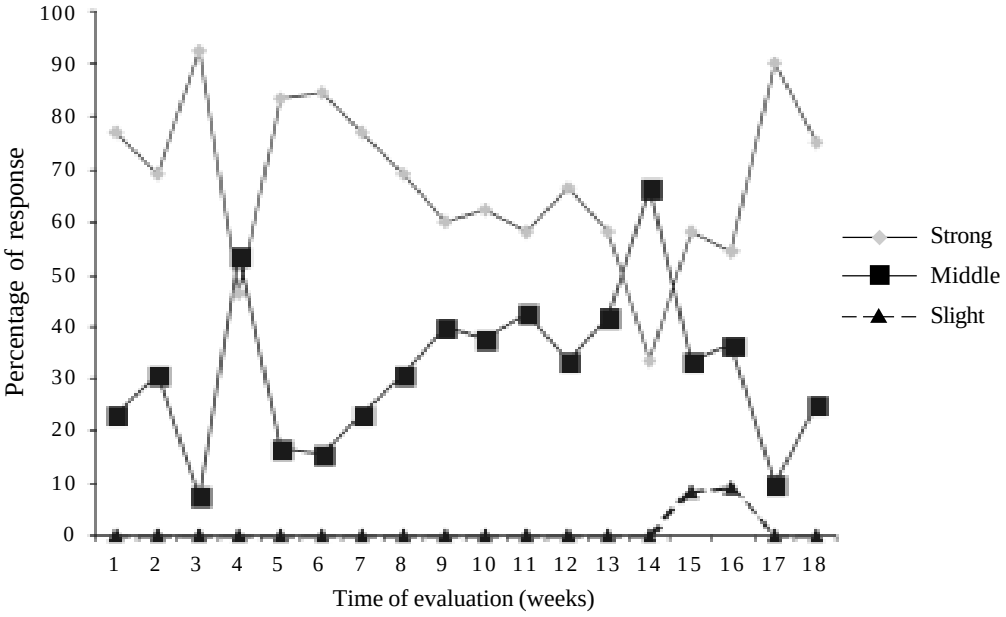


Figure 6. Follow-up of responses to Seminal Vesicle Reflex by rectal palpation in young males from the University of Antioquia BON herd.[a]

[a]Data are presented as the average response from all young males at each weekly point of evaluation.

size, climate and bred and feeding schedules (3, 27), which prevents extrapolation of data from a single study. The herd from University of Antioquia was bred in a humid subtropical forest (1), with a predominant plain soil (1400 m.o.s.l.); on the contrary, the herd from Corpoica was bred in a transition zone between humid tropical forest and very humid-tropical forest (800 to 1200 m.o.s.l.), with a predominant mountainous soil (13). Because of these differences and the imminent sale in 1998 of "El Progreso Farm", in which the BON herd of University of Antioquia was bred for several decades, the present work was designed to evaluated the onset and establishment of puberty in young males of this herd. The limited possibility to study young males for a long period (Weekly, 5 months), represents the major difference with our contemporary study carried out with the BON herd from Corpoica (Every-two weeks, 12 months) (13), regardless 19 and 24 time points of data collection, respectively, were included in the analysis. Because of the wide range of age between the youngest and the older male at each time point of evaluation found in both studies, which disables its analysis as repetitive measures, data obtained at each date were independently included and categorized into its respective group of age (For the present study see scatter plots from simple regression analysis in figures 1 and 2). Three key parameters are reported to define puberty onset in male bovine cattle: Body weight (BW), Scrotal circumference (SC), and semen concentration, with the last one being considered the most important (6-9, 11, 12). Most breeds show high correlation coefficients between these traits and age, reflecting the close relationship between precocity and the onset of puberty (12, 14). In addition, quantification of circulating LH and Testosterone (T) complements the battery of procedures for this purpose (10, 16-18, 25). Recently, important advances have been achieved in comprehending the mechanisms responsible for puberty onset in cattle, mainly in heifers (20, 23, 36). Increased frequency and amplitude of pulsate hypothalamic Gonadotropin-Releasing hormone (GnRH), appears to be the common mechanism for males and females (26). However, little is known about the precise mechanisms which associate BW and precocity with puberty onset (14, 18). Recent studies have correlated protein and energy levels in diet which modify the pattern of pulsate GnRH and affect puberty onset (5, 29, 36). Although these type of analysis were not made in our study, they could be considered for further studies in young males of BON cattle.

Table 2. Body traits in young males from the University of Antioquia BON herd, throughout puberty onset.*

Group of age (months)	n	Body weight (kg)	n	Body Lenght** (cm)	n	Wither height (cm)
10-11	8	251 ± 7^{a}	8	118 ± 3^{a}	8	112 ± 2^{a}
11,1-12	13	258 ± 7^{a}	10	119 ± 2^{a}	10	111 ± 2^{a}
12,1-13	12	271 ± 10^{a}	8	117 ± 3^{a}	9	111 ± 1^{a}
13,1-14	8	251 ± 4^{a}	7	115 ± 1^{a}	8	113 ± 1^{a}
14,1-15	9	267 ± 3^{a}	9	115 ± 1^{a}	9	$109 \pm 0,2^{a}$
15,1-16	7	288 ± 11^{a}	7	119 ± 1^{a}	7	110 ± 1^{a}
16,1-17	7	314 ± 10^{b}	4	114 ± 3^{a}	4	109 ± 1^{a}
17,1-18	5	313 ± 8^{b}	5	120 ± 4^{a}	5	114 ± 3^{a}
18,1-19	10	305 ± 8^{b}	5	126 ± 2^{b}	5	113 ± 1^{a}
19,1-20	13	312 ± 7^{b}	12	119 ± 2^{a}	12	115 ± 1^{b}
20,1-21	10	337 ± 3^{b}	7	120 ± 2^{a}	8	113 ± 2^{a}
21,1-22	10	359 ± 6^{c}	9	121 ± 2^{a}	9	115 ± 2^{b}
22,1-23	23	377 ± 6^{c}	9	128 ± 2^{b}	9	117 ± 2^{b}
23,1-24	30	397 ± 5^{c}	26	128 ± 1^{b}	26	125 ± 1^{b}
24,1-25	31	396 ± 4^{c}	31	134 ± 1^{c}	29	123 ± 1^{b}
25,1-26	20	396 ± 5^{c}	19	135 ± 1^{c}	19	124 ± 1^{b}
26,1-27	16	433 ± 5^{d}	14	131 ± 1^{b}	14	121 ± 1^{b}

*Least square means ± standard error

**Measured along the dorsal line from wither to the first *coccigeum vertebrae*

n = number of data in each group of age

Different superscripts within the same column indicate statistically significant differences ($p < 0,01$).

In order to optimize the detection of minimal variation in physical, testicular, behavioral and seminal traits, which could suggest puberty onset-related responses, we followed the method of the Breeding Soundness Examination (BSE) proposed for mature bulls (9, 31, 35) and nowadays applied to young males (11, 33), in order to achieve a more integral approach to puberty onset. In the present study we shall refer to puberty onset and establishment as the time of initiation of and transition to puberty and sexual maturity, respectively (8, 16, 21, 22).

Body traits

Body weight and SC showed the higher correlation coefficient with age, in agreement with reports for both *B. taurus* (6-8) and *B. indicus* (11, 33) cattle. Individual values for the period of study ranged between 232,5 and 448 kg for the youngest (male 23-7, 9,9 months of age) and the eldest (male 11-6, 28,1 months of age) males of the group (Table 1). Similar results were observed if the analysis was made by group of ages, when the youngest (10 to 11) and eldest (26 to 27 months), groups of age weighted 251 ± 7 and 433 ± 5 kg, respectively. Values found for the same groups of age in a contemporary study evaluating the BON herd of Corpoica, were lower than those here reported (13), clearly reflecting differences between both herds for its average size and weight, possibly as a result of different nutritional plans and climate variations (1, 34), as reported elsewhere (11). However, a high correlation between BW and age was found in both studies, which means that the faster period of growth elapsed between puberty onset and establishment was comprised, as found for other *B. indicus* and *B. taurus* cattle (11, 14).

Body length was included in the study with the aim of contributing to define reference values for BON cattle. Although this is a parameter not usually included for evaluating puberty onset, our results showed a mild correlation with age between both BL and HW (Figure 1B and 1C). Unfortunately, no reports could be found in the literature on the relationship of BL, and HW with puberty onset. One phenotypic characteristic of BON cattle is its relative low size, as corroborated in this study, in which only a difference of 12 cm were found for WH in a range of 14 months of age (Table 2). Neither BL nor WH were measured in the study with BON herd of Corpoica (13). However, it is important to clarify that BL is currently measured from

Table 3. Testicular traits of young males from the University of Antioquia BON herd, throughout puberty onset.*

Group of age (months)	n	Testicular diameter (kg)	n	Testicular lenght (cm)	n	Scrotal circumference (cm)
10-11	8	$4,9 \pm 0,16^{a}$	8	$10,1 \pm 0,5^{a}$	8	$26,4 \pm 0,2^{a}$
11,1-12	13	$4,9 \pm 0,18^{a}$	13	$9,6 \pm 0,3^{a}$	12	$26,7 \pm 0,3^{a}$
12,1-13	12	$5,5 \pm 0,18^{b}$	12	$11,0 \pm 0,4^{b}$	10	$27,2 \pm 0,4^{b}$
13,1-14	8	$5,6 \pm 0,17^{b}$	8	$11,3 \pm 0,3^{b}$	8	$28,4 \pm 0,5^{b}$
14,1-15	9	$6,0 \pm 0,15^{c}$	9	$11,7 \pm 0,4^{b}$	9	$30,1 \pm 0,2^{b}$
15,1-16	7	$6,2 \pm 0,08^{c}$	7	$11,9 \pm 0,4^{b}$	7	$31,3 \pm 0,1^{b}$
16,1-17	7	$6,5 \pm 0,10^{c}$	7	$13,1 \pm 0,3^{b}$	7	$31,4 \pm 0,5^{b}$
17,1-18	5	$6,2 \pm 0,08^{c}$	5	$13,1 \pm 0,2^{b}$	5	$33,7 \pm 0,4^{b}$
18,1-19	10	$6,5 \pm 0,12^{c}$	9	$14,1 \pm 0,6^{b}$	10	$32,1 \pm 0,9^{b}$
19,1-20	13	$6,0 \pm 0,14^{c}$	13	$12,9 \pm 0,3^{b}$	12	$31,4 \pm 0,9^{b}$
20,1-21	10	$6,0 \pm 0,24^{c}$	10	$12,6 \pm 0,3^{b}$	9	$30,6 \pm 0,5^{b}$
21,1-22	10	$5,9 \pm 0,22^{c}$	10	$12,3 \pm 0,2^{b}$	10	$30,7 \pm 0,4^{b}$
22,1-23	23	$6,6 \pm 0,12^{c}$	23	$13,7 \pm 0,3^{b}$	23	$32,9 \pm 0,5^{b}$
23,1-24	30	$6,7 \pm 0,12^{d}$	29	$13,8 \pm 0,2^{c}$	28	$34,3 \pm 0,3^{c}$
24,1-25	31	$6,7 \pm 0,10^{d}$	31	$13,8 \pm 0,2^{c}$	31	$35,4 \pm 0,3^{c}$
25,1-26	20	$6,7 \pm 0,13^{d}$	20	$13,9 \pm 0,3^{c}$	18	$35,5 \pm 0,5^{c}$
26,1-27	16	$6,8 \pm 0,10^{d}$	16	$13,9 \pm 0,2^{c}$	15	$35,7 \pm 0,4^{c}$
>27	5	$6,8 \pm 0,20^{d}$	5	$14,0 \pm 0,3^{c}$	5	$35,9 \pm 0,4^{c}$

*Least square means ± standard error

n = number of data in each group of age

Different superscripts within the same column indicate statistically significant differences ($p < 0,01$).

Table 4. Individual responses to seminal vesicle palpation of young males from University of Antioquia BON herd, throughout puberty onset.*

Male identification[a]	n	No response (%)	Preseminal fluid (%)	Protrusion of penis (%)	Complete erection (%)	Combined response[b] (%)
23-7	11	81,8	-	-	18,2	-
21-7	11	63,6	-	18,2	18,2	-
17-7	19	63,1	21	-	26,3	10,5
15-7	19	57,8	31,6	-	21	10,5
11-7	19	26,3	57,7	5,26	57,7	47,3
5-7	11	27,3	9,1	9,1	63,6	9,1
1-7	18	72,2	11,1	5,6	16,7	5,6
37-6	18	66,6	22,2	11,1	11,1	11,1
29-6	18	72,15	11,1	-	16,6	-
27-6	18	33,3	11,1	11,1	55,5	11,1
25-6	19	26,3	-	15,8	57,76	-
23-6	19	10,5	21	10,5	68,4	10,5
21-6	11	27,3	9,1	-	72,7	9,1
17-6	10	20	-	10	70	-
11-6	11	18,2	27,3	9,1	72,7	27,3

*Intensity of responses from left (No response) to right (Combined responses), proportionally increased with age ($R^2 = 0,539$; $p < 0,01$).
[a]Males are ordered chronologically from youngest (Born in 1997) to older (Born in 1996) at the beginning of evaluations.
[b]All combined responses indicate pre-seminal fluid plus complete erection.

withers to the major femoral trocanter, but in our study it was measured from withers to the first *coccigeum vertebrae*.

Reduction in growth rates observed for BW between 12 and 13, and 17 to 18 months (Figure 3), as well as for WH between 18 to 19 and 18 to 19 months (Figure 4) could not be explained, but they could be related to physiological adaptations of males to puberty onset and transition to sexual maturity (35). Even though it is possible to consider that the low number of observation for each group could interfere with significance of the data, the relatively low standard error (Table 2) suggest that this variations were indeed a physiological response of the males. In addition, the BL:WH ratio ranged between 1,0 and 1,1, reflecting a similar growth rate for these traits for the period of study (Data not shown).

Testicular traits

Values for SC have been reported to be 28 cm at the onset of puberty for most *B. taurus* cattle, which was found at 13 to 14 months of age for this BON herd (Table 3). These values are slightly similar to those found in the study with the BON herd of Corpoica, in which males had 28 cm of SC at 15 months (13). These results are in agreement with those reported for other tropically-adapted *B. taurus* breeds (11, 35). In the present study, SC had the higher correlation with age, though TL and TD, had a mild correlation (Figure 2). Interestingly, the TL:TD ratio remain near to 2,0 and the SC:TL ratio range between 2,7 and 2,5 throughout the range of age evaluated (Data not shown). Growth of testis in terms of outer (stromal) and inner (germinal) cells in seminiferous tubes, appears to be the one of the main changes associated with puberty onset, in response to increasing concentration of gonodotropic (LH, FSH) and steroids (Testosterone and its metabolites) hormones, acting on Sertoli, Leydig and stromal cells (18, 25, 35). In the present study, TL and TD reached a plateau at the eldest groups of age, whereas SC remains growing (See polynomial transformation of data in figure 5). It seemed that other factor or another trait (for example left testis size, or testis volume) which was not included in the present study, could explain the different growth rates found between TD, TL and SC. In addition, the differences in correlation coefficients found for these testicular traits could be explained because measurement of SC included both testis, but only the

Table 5. Semen analysis of samples yielded by young males from the University of Antioquia BON herd, after puberty onset.

Group of age (months)	n	Volume[a] (ml)	pH[a]	Concentration[a] (10^6/cc)	Individual and gross motility VG (%)	G (%)	P (%)
16,1-21	10	3,0 ± 0,3	6,88 ± 0,1	126 ± 9	60	30	10
21,1-24	8	3,9 ± 0,6	6,87 ± 0,1	125 ± 11	62,5	37,5	0
24,1-27	21	3,8 ± 0,3	6,85 ± 0,07	111 ± 8	62	33,3	4,7

[a]Least square means ± standard error
n = number of data for each group of age
VG: very good
G: good
P: poor

right testis was measured for TD and TL. In a report by Gabor et al, correlations were made between these traits in mature Senepol (B. taurus) bulls, which showed a linear relationship between SC and testicular weight, TD and TL, but a curvilinear relationship between SC and BW (19).

Seminal Vesicle Reflex and Libido testing

Because the reproductive function in bulls is related in part to sexual desire and mating ability, we thought to profit the evaluation of SVR and LibT, a common practice in the BSE of mature bulls, in order to achieve a more comprehensive approach on the effect of this component to define puberty onset in young BON males. In addition, breeders (particularly in our BON herd) well known about the intense sexual desire of young BON males, some times since calves. In fact, the predominant percentage of strong responses for SVR found in the study could reflect and confirm the early (maybe subclinical) onset of puberty in this BON herd. Physiological concentrations of testosterone are responsible to induce both behavioral and physical changes (4, 25), necessaries for exhibiting Libido, secreting pre-seminal fluid, protrusion of penis and complete erection. It is noteworthy that BON males exhibited very good Libido scores at all: the first time point when young males were evaluated, all of them exhibited more than five service attempts/5 minutes (which overpass the maximum classification proposed by Barth (6), although slight variations were observed by the presence of the dominant male in each group of exposition as demonstrated in other study (24). Furthermore, the responses of SVR correlates with the responses in terms of production of pre-seminal fluid, penis protrusion and complete erection, which increased with age. These results confirm the common observation of sexual precocity in BON males and suggest a "behavioral" beginning of puberty at an early time before the males reach the reference value for puberty onset defined by semen concentration (5 x 10^7 sperm/cc with at least 10% of progressive motility). However, its is possible that pre-pubertal brain-derived rather than gonadal-derived testosterone or its metabolites (4, 22, 25) could be responsible for this particular behavior in BON cattle. Further studies should ruled out this possibility. Interestingly, this kind of evaluation are not commonly reported because palpation of seminal vesicle is mainly focused in detecting abnormalities in mature bulls rather than studying physiological responses. Moreover, we propose an additional tool to evaluate seminal vesicle and its correlation with reproductive potential.

Semen analysis

The onset of puberty is defined as the time when the first ejaculate have 5 x 10^7 sperm/cc and 10% progressive motility (6, 9). However, at the age of first successful semen collection (16,1 months), the ejaculate had 1,63 x 10^8 sperm/cc (Table 5), which prevents the use of this parameter to define puberty onset. In this study young males were not previously trained for semen collection with artificial vagina, and the real age when males had 5 x 10^7 sperm/cc was surpassed. Besides other characteristics of semen analysis such as gross and individual progressive motility, showed good scores (more than 60%) for all samples evaluated, suggesting that puberty onset had occurred several weeks ago, before the youngest age at first collection. According to Coulter, 60% of progressive motility can be found approximately 8

weeks after puberty onset (14), which corresponded to 14 months in our study.

Persistent frenulums (7, 12), interferes with the disposition of young males to ejaculate when artificial vagina is used, instead of producing low quantities of semen and secreting accessory gland fluids early during puberty onset. Bruner et al (7) found an incidence rate of persistent frenulums of 4.4% in yearling bulls. Unfortunately, we did not evaluate its incidence in our study. In our contemporary study (13) it was common to observe young males having high scores for LibT, but not capable of ejaculate as a consequence of the penis remaining adhered to the sheath. For instance, this finding must be an obligatory trait to evaluated in further studies, particularly in BON cattle regarding its apparently early "behavioral" onset of puberty.

The yellowish color and aqueous aspect found in most of semen sample collected, resembled the low concentration of semen typically yielded at puberty onset (14). However, a controversy between semen concentration (relatively low) and scores for gross and individual progressive motility (good and very good) could be argued, but these results were consistently reported by trained technicians.

Measurement of circulating levels of Testosterone (T) or LH would help to define the precise onset of puberty in our BON males as recommended by Chenoweth (10). However, because of the low correlation coefficient found between testosterone and puberty onset in the contemporary study with the BON herd of Corpoica (13), we did not measured testosterone concentrations. In addition, it is clear that GnRH and LH frequency pulses, rather than testosterone concentrations closely predicts the onset of puberty. Accordingly, LH and T measurements and the use of electroejaculator and testicular ultrasonografic and thermographic studies (19), will allow a more precise definition of puberty onset in BON cattle.

Future implications

This study and the study by Cordoba et al (13) represent the first approaches to define puberty onset in young males from BON cattle in Colombia. Instead of slight differences found in both studies, the overal results suggest the onset of puberty between 13-14 months in this breed. However, further studies should include measurements of LH and T between 10 and 14 months, in order to more precisely define puberty onset and to study other physiological-related processes such as contribution of brain- and gonadal-derived steroids to high libido exhibited by pre and per-pubertal BON males. These results suggest that BSE rather than merely semen concentration must be considered in order to define puberty onset in bovine cattle.

Resumen

Medidas corporales y testiculares, prueba de libido y análisis de semen en machos jóvenes blanco oreginegro (Bos taurus) de la Universidad de Antioquia, al comienzo de la pubertad.

Quince toretes (de diferentes edades) del hato BON de la Universidad de Antioquia fueron evaluados cada semana, desde agosto hasta diciembre de 1998 (incluyendo un rango de edad de 9,9 a 28,1 meses, en 19 fechas de medición), para evaluar: 1) Medidas corporales y testiculares; 2) Prueba de la líbido (LibT); 3) Reflejo a la palpación de las vesículas seminales (SVR, n = 244) por masaje rectal; y 4) Análisis de muestras de semen (n = 42) obtenidas mediante vagina artificial. Los datos de medidas corporales y testiculares fueron categorizados en grupos de un mes (desde 10 hasta 27 meses; n = 17 grupos); y para el análisis del semen se categorizaron en tres grupos (16,1 a 21, 21,1 a 24, y 24,1 a 27 meses). Pruebas de regresión simple se hicieron incluyendo cada parámetro como variable dependiente y la edad como variable independiente. La prueba de ANOVA Multifactorial se utilizó para definir diferencias y valores de referencia para cada parámetro. Las medidas corporales y testiculares aumentaron proporcionalmente con la edad ($p < 0,01$): el peso corporal ($R^2 = 0,796$) y la circunferencia escrotal ($R^2 = 0,672$) mostraron la más alta correlación con la edad, mientras que mostraron una mediana correlación la longitud corporal ($R^2 = 0,454$), la alzada ($R^2 = 0,506$), el diametro del testículo ($R^2 = 0,415$), y la longitud del testículo ($R^2 = 0,458$). En la LibT, la mayoría de los toretes mostraron más de cinco intentos de monta/5 minutos. La intensidad de la respuesta individual al masaje rectal (secreción de líquido preseminal, protrusión del pene y erección completa), aumentó proporcionalmente con la edad ($R^2 = 0,539$; $p < 0,01$). El primer eyaculado se recolectó a la edad de 16,1 meses ($1,63 \times 10^8$

espermatozoides/cc, con mas de 60% de movilidad progresiva en masa e individual); sin embargo, no se halló correlación entre el volumen (3,6 ml), el pH (6,86), o la concentración del semen, con la edad. Los resultados sugieren un inicio de la pubertad entre 13 y 14 meses de edad para el grupo de toretes de este hato. Ademas, se informan los valores de referencia para cada medida corporal y testicular, en el rango de edad evaluado.

Palabras clave: *Bovinos, Ganado criollo, Pubertad, Semen, Toretes, Trópico.*

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References

1. Anuario Estadístico de Antioquia, 1996. República de Colombia, Gobernación de Antioquia, Departamento Administrativo de Informática y Estadística.
2. Arboleda O, Gutiérrez ID. El ganado blanco orejinegro BON. Rev Col Cienc Pec 1996 (Suppl); 80-83.
3. Arboleda O. El ganado blanco orejinegro. Suplemento Ganadero 1980; 1:5-41.
4. Aspden WJ, Rodgers RJ, Stocco DM, Scott PT, Wreford NG, Trigg TE, Walsh J, D'Occhio MJ. Changes in Testicular Steroidogenic Acute Regulatory (STAR) protein, steroidogenic enzymes and testicular morphology associated with increased testosterone secretion in bulls receiving the luteinizing hormone releasing hormone agonist deslorelin. Domestic Anim Endocrinol 1998; 15:227-238.
5. Azage Tegegne, Entwistle KW, Mukasa-Mugerwa E. Gonadal and extragonadal sperm reserves and testicular histometric characteristics in Zebu and crossbred bulls: effect of dry season nutritional supplementation. Anim Reprod Sci 1992; 29:25-33.
6. Barth AD. Evaluation of potential breeding soundness of the Bull. In: Youngquist RS (Ed). Current therapy in large animal theriogenology. Saunders, Philadelphia; 1997. p221-236.
7. Bruner KA, McCraw RL, Whitacre MD, Van Camp SD. Breeding soundness examination of 1,952 yearling beef bulls in North Carolina. Theriogenology 1995; 44:129-145.
8. ChaseJr CC, Chenoweth PJ, Larsen RE, Olson TA, Hammond AC, Menchaca MA, Randel RD. Growth and reproductive development from weaning through 20 months of age among breeds of bulls in subtropical Florida. Theriogenology 1997; 47:723-745.
9. Chenoweth PJ, Ball L. Breeding Soundness Evaluation in Bulls. In: Morrow DA (Ed). Current therapy in theriogenology. Saunders, Philadelphia; 1989. 136-146.
10. Chenoweth PJ, Brinkks JS, Nett M. Comparison of three methods to assessing sex drive in yearling beef bulls and relationship with testosterone and LH levels. Theriogenology 1979; 12: 223-224.
11. Chenoweth PJ, ChaseJr JJ, Thatcher M-JD, Wilcox CJ, Larsen RE. Breed and other effects on reproductive traits and breeding soundness categorization in young beef bulls in Florida. Theriogenology 1996; 46:1159-1170.
12. Chenoweth PJ. Clinical reproductive anatomy and physiology of the bull. In: Youngquist RS (Ed). Current therapy in large animal theriogenology. Saunders, Phipladelphia; 1997. p217-222.
13. Cordoba SL, Maldonado JG, Trujillo LE, Tobon JA, Neira JA. Determinación de la edad y peso al inicio de la pubertad en machos de la raza criolla colombiana Blanco Orejinegro, BON (Submitted: Rev Corpoica).
14. Coulter GH. Puberty and postpuberal development of beef bulls. In: Youngquist RS. Current therapy in large animal theriogenology. Saunders, Phipladelphia; 1997. p142-148.
15. Deer JN, Davis SK, Estrada JL, et al. Genetic characterization and conservation of colombian criollo cattle. In: Crowford RD, Lister EE, Buckley JT (Eds). Proceedings of the third global conference on conservation of domestic animal genetic resources. Rare Breeds International; Queens University, Kingston, 1995; p307-313.
16. Evans ACO, Davies FJ, Nasser JF, Bowman P, Rawlings NC. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. Theriogenology 1995; 43:569-578.
17. Evans ACO, Pierson RA, Garcia A, McDougall LM, Hrudka F, Rawlings NC. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. Theriogenology 1996; 46:345-357.
18. Forest M, Levasseur MC. La puberté. Chapter 28. In : Thibault C, Levasseur MC (Eds). La reproduction chez les mammifères et l'homme. INRA-Ellipses, Joy-en-Josas, 1991; p551-571.
19. Gabor G, Sasser RG, Kastelic JP, Coulter GH, Falkay Gy, Mézes M, Bozó S, Völgyi-Csík J, Bárány I, Szász Jr. F.

Morphologic, endocrine and thermographic measurements of testicles in comparison with semen characteristics in mature Holstein—Friesian breeding bulls. Anim Reprod Sci 1998; 51:215-224.

20. Gaillard RC, Spinedi E. Sex- and stress-steroids interactions and the immune system: evidence for a neuroendocrine-immunological sexual dimorphism. Domestic Anim Endocrinol 1998; 15:345-352.
21. Garner DL, Johnson LA, Allen CH, Palencia DD, Chambers CS. Comparison of seminal quality in Holstein bulls as yearlings and as mature sires. Theriogenology 1996; 45:923-934.
22. Jiménez-Severiano H, Jiménez-Krassel F, Menéndez-Trejo M. Endocrine and testicular responses of prepubertal Zebu crossbred bulls to active immunization against testosterone. Anim Reprod Sci 1996; 41:169-181.
23. Knickerbocker JJ, Drost M, Thatcher WW. Endocrine patterns during the initiation of puberty, the estrous cycle, pregnancy and parturition in cattle. In: Morrow DA. Current Therapy in Theriogenology. Saunders, Philadelphia; 1986. p117-121.
24. López H, Orihuela A, Silva E. Effect of a dominant bull on performance of two age group bulls in libido tests. Anim Reprod Sci 1999; 65:13-20
25. Mainwaring WIP, Haining SA, Haerper B. The function of testosterone and its metabolites. In: Cooke BA, King RBJ, van der Molen HJ (Eds). Hormones and its actions. Part 1. Elsevier, New York; 1988. p169-196.
26. McCann SM, Kimura M, Walczewska A, Karanth S, Rettori V, Yu WH. Hypothalamic control of gonadotropin secretion by LHRH, FSHRF, NO, cytokines, and leptin. Domestic Anim Endocrinol 1998; 15:333-344.
27. Moreno F, Ruíz-Linares A. El concepto de genética de poblaciones y el proyecto genoma bovino. Rev Col Cienc Pec 1996 (Suppl); 77-80.
28. Ossa JE. Caracterización genética y conservación del ganado criollo colombiano. Rev Col Cienc Pec 1996 (Suppl); 85.
29. Rocha A, Carpena M, Triplett B, Neuendorff RA, Forrest DW, Randel RD. Testicular and epididymal function during the peripuberal period in Brahman bulls receiving various amounts of protein degradable in the rumen. Theriogenology 1996; 45:477-488.
30. Rodríguez L, Valencia L, Ruíz ZT, Andrade JC, Ochoa J, Vélez MP, Maldonado JG. Respuesta superovulatoria de vacas criollas colombianas blanco orejinegro (BON) al tratamiento con FSH o PMSG: informe de tres casos. Rev Col Cienc Pec 1998; 11:37-44.
31. Schramm RD, Osborne PI, Thaayne WV, Wagner WR. Inskeep EK. Phenotypic relationship of scrotal circumference to frame size and body weight in performance-tested bulls. Theriogenology 1989; 31:495-504.
32. Sierra RA, Berdugo JA, Cuartas JF, Aguirre VA, Olivera-Ángel M. Caracterización espermática del ganado Blanco orejinegro (BON) y cebú brahman para la producción de embriones bovinos in vitro (IVP): proyecto de investigación. Rev Col Cienc Pec 1998 ; 11:50-52.
33. Silva-Mena C. Peripubertal traits of Brahman Bulls in Yucatan. Theriogenology 1999; 48:675-685.
34. Tobón CJ, Franco CE, Mejía A. Evaluación de caracteres de crecimiento y reproductivos de la raza blanco orejinegro BON. Cebú, y sus cruces en zona de ladera. Rev Col Cienc Pec 1996 (Suppl); 84-85.
35. Wildeus S. Age-related changes in scrotal circumference, testis size and sperm reserves in bulls of the tropically adapted Senepol breed. Anim Reprod Sci 1993; 32:185-195.
36. Yelich JV, Wettemann RP, Marston TT, Spicer LJ. Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. Domestic Anim Endocrinol 1996; 13:325-338.