



## Sexual development of Guzerat (*Bos taurus indicus*) bulls raised in a tropical region

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### Abstract

Two-hundred and seven healthy males, 7 to 31 months of age and raised on pasture in a tropical region, were used to evaluate sexual development in the Guzerat breed (*Bos taurus indicus*). Body weight, body condition, testicular measurements and semen quality were recorded. Semen collection attempts were performed by electro-ejaculation at 3-months intervals, starting at 14 months of age. Thirty-eight sexually inexperienced males were used to evaluate the exhibition of sexual behavior at two age ranges: 12.1 to 17.4 months of age (G1) and 18.4 to 28.4 months (G2). Each male was evaluated individually (IND) or in groups under competition (COM); (5 males per group) free in a pasture with 5 cows (3 in heat and 2 not in heat). Body weight increased linearly from 7.5 to 30.7 months of age. Scrotal circumference and testicular diameter increased linearly with age and body weight. Testicular length had a quadratic growth with age and weight. First motile sperm cells (considered as puberty) were detected at the 18.2±2.1 months of age, 255.5±32.0 kg body weight and 24.2±2.4 cm of scrotal circumference. The percentage of bulls that exhibited sexual behavior was higher in the G2 than in the G1 group for both IND and COM evaluations. The number of sexual events displayed by active males did not differ among age groups. It was concluded that Guzerat bulls, raised in pasture under tropical conditions, reached puberty around 18 months of age, and did not reach testicular growth plateau until 30 months. Expression of sexual behavior started during the prepubertal period and differences between individuals were already detectable during this stage of development.

**Keywords:** Zebu cattle, bulls, Guzerat, puberty, reproductive development, sexual behavior.

### Introduction

The selection of superior sires as early as possible is desired for natural mating or for artificial insemination programs. In countries where extensive management systems are frequently used, particularly in tropical regions, natural mating is still being extensively applied to large herds. The early detection of sires with high breeding potential (i.e., high libido and sperm quality) is extremely important to improve productivity in extensive management systems (Peña- Alfaro *et al.*, 2001) and to establish the most appropriate period for the beginning of mating activities (Godinho, 1970).

Puberty is the first cue of reproductive capacity

and sexual maturity is the expression of maximum reproductive potential (Makarechian *et al.*, 1985). The combination of libido and sperm production during sexual development leads to reproductive capacity (Chenoweth *et al.*, 1984; Fonseca, 1989; Perry *et al.*, 1991; Price and Wallach, 1991b).

Many studies have characterized development of reproductive traits in *Bos taurus taurus* breeds (Jiménez-Severiano, 2002; Landaeta-Hernández *et al.*, 2001). Libido of young bulls is essential for fertilization of females and still deserves further investigation in *Bos taurus taurus* (Boyd *et al.*, 1991) and particularly in *Bos taurus indicus* breeds. Puberty and sexual maturity occur later in *Bos taurus indicus* bulls when compared to *Bos taurus taurus* bulls (Vale-Filho *et al.*, 1986; 1993) and most studies in *Bos taurus indicus* breeds have used the Nelore breed.

In the Guzerat breed (*Bos taurus indicus*), testicular development (Valvasori *et al.*, 1985; Garcia *et al.*, 1987; Pinto *et al.*, 1989; Cartaxo *et al.*, 2001) and particularly sexual behavior expression in males during the peripubertal period has been poorly studied. Further research is needed to have reliable data in order to properly select young sires of the breed. Therefore, the objectives of the present study were to contribute to the characterization of testicular growth and sperm production development along with expression of sexual behavior for use in selection programs for young Guzerat bulls raised on pasture in tropical regions.

### Materials and Methods

The present experiment was conducted in a tropical region at 17°32'54" S and 46°13'227" W. The average temperature during the winter (dry period) was 20.7°C (15.3 to 27.2° C) and 24.0°C (19.7 to 29.1°C) in summer (rainy season; Anuário, 2002). Annual rain falls were concentrated between November and March. A total of 207 healthy and sexually inexperienced Guzerat (*Bos taurus indicus*) bulls were used. Animals were raised in a single group on pasture (*Brachiaria brizantha*) and supplemented with minerals *ad libitum*.

Body weight (BW), body condition (BC), scrotal circumference (SC), testicular length (TL) and diameter (TD) were recorded at 3-month intervals for 4 consecutive periods. During the evaluation period, the age of the bulls ranged from 7.5 to 30.7 months. Body condition was assessed by visual scores on a scale range from 1 (emaciated) to 5 (obese) according to Jorge Jr. *et al.* (2001). Bulls were restrained in a squeeze chute and

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testes were measured before electro-ejaculation. Scrotal circumference was measured *in situ* with a scrotal tape and proximal-distal length and cranial-caudal diameter of each testis were measured with a caliper. Testicular measurements were used to estimate volume using the following equation:  $V = 2[(r^2) \cdot \pi \cdot h]$  where  $r$  = diameter/2,  $h$  = length,  $e \pi = 3.14$  (Fields *et al.*, 1979). An attempt of semen collection by electro-ejaculation was performed in all animals at each examination. In case of an unsuccessful attempt, a second stimuli session was always tried immediately after the first one. Sperm progressive motility and vigor (from 0 - no- movement to 5 - very fast spermatozoa movements) were evaluated immediately after collection. Sperm morphology was observed using a phase contrast microscope evaluating 200 cells per sample. Age at the onset of puberty was considered to be the age of the first successful sperm collection attempt.

The exhibition of sexual behavior was studied in thirty eight sexually inexperienced bulls divided into two age groups: G1 (12.1 to 17.4 months; n=19) and G2 (18.4 to 28.4 months; n=19). Evaluation was performed by the two following methods. Competition Evaluation (COM) - five males of similar body weights within an age group were allocated together with 5 cows for one hour. During this period, bulls were allowed to run free in a paddock (100x100m) out of the visual range of their group mates. A technician on horseback recorded the expression and duration of any sexual behavior. The individual Evaluation (IND) was performed immediately after each COM evaluation. Each bull of the group was allocated individually in the same pen with 5 cows. Duration and method of observation were similar to that used in the COM method. In both evaluation methods, the proportion of three cows in heat to two cows not in heat was carefully observed. All bulls tested were submitted to the same evaluation sequentially. Sexual interest was reported by the exhibition of the following behavior: smelling and/or

licking female body (SLB), smelling and/or licking female perineal region (SLP), Flehmen reflex (FR), female approach and pursuing (FA), chin pressure (CP), head strikes (HS), mount reflex (MR), mounting without penile exposition (MwPE), aborted mount (AM), and complete mount (CM).

Data are presented as means  $\pm$  standard deviations. Body and testicular measurements of different age groups were analyzed by one-way analysis of variance. The parametric variables (body weight, scrotal circumference, testicular length, testicular diameter and testicular volume) were compared by the Student-Newman-Keuls test and the nonparametric variables (body condition, progressive motility, vigor and percentage of spermatic abnormalities) were compared by the Kruskal-Wallis test. Regression analysis was used to characterize relationships between age, body weight, and testicular measurements (scrotal circumference, testicular length and testicular diameter). Correlation coefficients between variables were calculated. Comparison of the percentage of bulls exhibiting each sexual behavior between methods of evaluation was done by the Fisher's exact test and mean frequency of each sexual behavior shown by active bulls in both evaluation methods was presented only in a descriptive way. Values were considered significantly different when  $P < 0.05$ .

## Results

Body condition was affected by season ( $P < 0.05$ ). In December (beginning of the rainy season and the first evaluation period), bulls had a lower body condition that increased gradually in subsequent evaluation periods. A lower body condition ( $P < 0.05$ ) was observed in bulls ranging from 7.5 to 12.9 months of age. Weight loss was not observed between observation periods and the average daily gain was  $0.45 \pm 0.26$  kg/day and did not differ between age groups ( $P > 0.05$ ).

Table 1. Body and testicular traits (mean  $\pm$ SD) of young Guzerat grouped by age.

	Age (months)							
	7.5-9.9	10.0-12.9	13.0-15.9	16.0-18.9	19.0-21.9	22.0-24.9	25.0-27.9	28.0-30.7
(n)	(49)	(92)	(76)	(71)	(87)	(86)	(29)	(17)
Traits								
Age	8.9 $\pm$ 0.8 <sup>h</sup>	11.4 $\pm$ 0.9 <sup>g</sup>	14.3 $\pm$ 0.9 <sup>f</sup>	17.2 $\pm$ 0.8 <sup>e</sup>	20.2 $\pm$ 0.8 <sup>d</sup>	23.6 $\pm$ 0.8 <sup>c</sup>	25.9 $\pm$ 0.6 <sup>b</sup>	29.3 $\pm$ 0.7 <sup>a</sup>
BC <sup>†</sup>	2.0 $\pm$ 0.5 <sup>D</sup>	2.3 $\pm$ 0.4 <sup>D</sup>	2.7 $\pm$ 0.5 <sup>C</sup>	3.3 $\pm$ 0.4 <sup>ABC</sup>	3.2 $\pm$ 0.4 <sup>BC</sup>	3.2 $\pm$ 0.5 <sup>C</sup>	3.7 $\pm$ 0.4 <sup>AB</sup>	3.8 $\pm$ 0.2 <sup>A</sup>
BW (kg)	130.4 $\pm$ 27.0 <sup>h</sup>	154.9 $\pm$ 26.1 <sup>g</sup>	193.9 $\pm$ 29.1 <sup>f</sup>	242.6 $\pm$ 27.2 <sup>e</sup>	280.1 $\pm$ 38.1 <sup>d</sup>	308.0 $\pm$ 40.7 <sup>c</sup>	359.5 $\pm$ 38.4 <sup>b</sup>	392.5 $\pm$ 31.6 <sup>a</sup>
SC (cm)	16.0 $\pm$ 1.8 <sup>g</sup>	17.2 $\pm$ 1.7 <sup>f</sup>	19.4 $\pm$ 2.8 <sup>e</sup>	23.1 $\pm$ 2.7 <sup>d</sup>	26.1 $\pm$ 3.3 <sup>c</sup>	29.1 $\pm$ 3.5 <sup>b</sup>	31.1 $\pm$ 3.0 <sup>a</sup>	32.7 $\pm$ 2.5 <sup>a</sup>
TL (cm)	4.5 $\pm$ 0.8 <sup>g</sup>	5.2 $\pm$ 0.8 <sup>f</sup>	5.9 $\pm$ 0.8 <sup>e</sup>	7.1 $\pm$ 1.0 <sup>d</sup>	8.3 $\pm$ 1.5 <sup>c</sup>	9.5 $\pm$ 1.3 <sup>b</sup>	10.1 $\pm$ 1.3 <sup>a</sup>	9.7 $\pm$ 0.8 <sup>ab</sup>
TD (cm)	2.8 $\pm$ 0.5 <sup>h</sup>	3.1 $\pm$ 0.4 <sup>g</sup>	3.7 $\pm$ 0.5 <sup>f</sup>	4.4 $\pm$ 0.6 <sup>e</sup>	5.0 $\pm$ 0.6 <sup>d</sup>	5.5 $\pm$ 0.7 <sup>c</sup>	6.2 $\pm$ 0.6 <sup>b</sup>	6.5 $\pm$ 0.5 <sup>a</sup>
TV (cm <sup>3</sup> )	58.5 $\pm$ 26.4 <sup>f</sup>	83.7 $\pm$ 31.3 <sup>f</sup>	131.4 $\pm$ 53.4 <sup>e</sup>	224.0 $\pm$ 92.9 <sup>d</sup>	346.0 $\pm$ 141.4 <sup>c</sup>	474.7 $\pm$ 167.8 <sup>b</sup>	621.5 $\pm$ 178.9 <sup>a</sup>	630.6 $\pm$ 152.7 <sup>a</sup>

Means within rows with different lower case superscripts differ  $P < 0.05$ ; Means within rows with different capital letters superscripts differ  $P < 0.05$ ; BC: body condition; BW: body weight; SC: scrotal circumference; TL: testicular length; TD: testicular diameter; TV: testicular volume; n: number of bulls per group; Body score -1 (emaciated) to 5 (obese).



Table 2. Percentage of Guzerat (*Bos taurus indicus*) bulls reaching puberty grouped by age and scrotal circumference.

Age groups	<13.9	13.9-15.9	16.0-18.9	19.0-21.9	22.0-24.9	25.0-30.7
%	0 (0 / 35)	16.6 (9 / 54) <sup>d</sup>	69.0 (49 / 71) <sup>c</sup>	87.4 (19 / 87) <sup>b</sup>	91.9 (79 / 86) <sup>b</sup>	100 (46 / 46) <sup>a</sup>
SC groups	<20	20.0-22.9	23.0-25.5	26.0-28.9	29.0-31.9	32.0-36.0
%	0 (0 / 58)	32.5 (25 / 77) <sup>d</sup>	79.7 (51 / 64) <sup>c</sup>	94.0 (63 / 67) <sup>b</sup>	95.4 (62 / 65) <sup>ab</sup>	100 (47 / 47) <sup>a</sup>

<sup>a,b</sup> Values within rows with different superscripts differ  $P<0.05$ .; SC = scrotal circumference; Puberty<sup>†</sup>: first age at which motile spermatozoa were observed in the ejaculate.

Body and testicular growth of the Guzerat bulls by age groups are shown in Table 1. Body weight, scrotal circumference, and testicular diameter increased linearly within the range of 7.5 to 30.7 months of age ( $Y=9.08+13.2X$ ,  $P<0.0001$ ,  $R^2=0.85$ ;  $Y=6.98+0.93X$ ,  $P<0.0001$ ,  $R^2=0.79$ ; and  $Y=0.956+0.198X$ ,  $P<0.0001$ ,  $R^2=0.79$ , respectively). In addition, a linear increase in scrotal circumference and testicular diameter was observed with body weight ( $Y=7.15+0.067X$ ,  $P<0.0001$ ,  $R^2=0.84$ ; and  $Y=1.02+0.014X$ ,  $P<0.0001$ ,  $R^2=0.82$ , respectively). There was a quadratic growth pattern between testicular length and age ( $Y=0.477+0.461X-0.003758X^2$ ,  $P<0.0001$ ,  $R^2=0.73$ ) or body weight ( $Y=0.307+0.035X-0.00002X^2$ ,  $P<0.0001$ ,  $R^2=0.74$ ) with a curvilinear trend after 21 months and greater than 250 kg of body weight. Correlations were significant between age, body weight, and all testicular measurements ( $r = 0.85$ ,  $P<0.0001$ ).

Testicular growth was lowest ( $P<0.05$ ) from 10.0 to 12.9 months age ( $0.13\pm0.13\text{mm/day}$ ). The increase in scrotal circumference was greater after 13 months of age, reaching a maximum growth rate

between 16.0 and 21.9 months ( $0.37\pm0.20\text{mm/day}$ ). There was a progressive reduction in testicular growth rate after 22 months of age, with an growth rate of  $0.1\pm0.1\text{mm/day}$  from 28.0 to 30.7 months.

The percentage of Guzerat bulls reaching puberty for different age and scrotal circumference groups are summarized in Table 2. First detection of motile sperm cells (puberty) in the ejaculate occurred at  $18.2\pm2.1$  (range, 13.9 to 24.2) months of age,  $255.5\pm32.0$  (from 176 to 335) kg of body weight), and  $24.2\pm2.4$  (from 20 to 31) cm of scrotal circumference. Physical and morphological seminal characteristics were evaluated as soon as sperm cells appeared in the ejaculate. Results for each age group are summarized in Table 3. Progressive motility, vigor, and percentage of normal sperm cells increased ( $P<0.05$ ) gradually with age. The proximal droplet was the predominant sperm abnormality detected during the pubertal and postpubertal periods. The second and third most frequently-observed abnormalities were midpiece defects and pyriform heads, respectively. The frequency of other sperm abnormalities was low.

Table 3. Seminal traits of Guzerat bulls between 7.5 and 30.7 months of age.

	Age (months)							
	7.5-9.9	10.0-12.9	13.0-15.9	16.0-18.9	19.0-21.9	22.0-24.9	25.0-27.9	28.0-30.7
n			(08)	(39)	(73)	(79)	(29)	(16)
Seminal traits								
Motility (%)	ND	ND	44.4 $\pm$ 21.3 <sup>b</sup>	47.4 $\pm$ 26.0 <sup>b</sup>	54.1 $\pm$ 24.2 <sup>b</sup>	49.9 $\pm$ 23.7 <sup>b</sup>	64.8 $\pm$ 24.8 <sup>ab</sup>	75.9 $\pm$ 15.2 <sup>a</sup>
Vigor <sup>†</sup>	ND	ND	1.4 $\pm$ 0.5 <sup>b</sup>	1.7 $\pm$ 0.9 <sup>b</sup>	1.9 $\pm$ 0.9 <sup>b</sup>	2.5 $\pm$ 1.0 <sup>a</sup>	3.0 $\pm$ 1.2 <sup>a</sup>	3.0 $\pm$ 0.8 <sup>a</sup>
Abnormal heads (%)	ND	ND	11.4 $\pm$ 4.9	8.7 $\pm$ 5.6	7.8 $\pm$ 8.5	5.2 $\pm$ 4.6	7.0 $\pm$ 7.3	6.1 $\pm$ 4.8
Abnormal tails (%)	ND	ND	15.0 $\pm$ 9.4 <sup>ab</sup>	18.7 $\pm$ 12.3 <sup>a</sup>	13.3 $\pm$ 8.9 <sup>ab</sup>	9.5 $\pm$ 6.9 <sup>b</sup>	9.0 $\pm$ 5.9 <sup>b</sup>	9.1 $\pm$ 8.3 <sup>b</sup>
Proximal droplets (%)	ND	ND	60.0 $\pm$ 14.9 <sup>a</sup>	36.4 $\pm$ 23.3 <sup>a</sup>	29.0 $\pm$ 27.0 <sup>a</sup>	12.1 $\pm$ 22.1 <sup>b</sup>	4.3 $\pm$ 9.8 <sup>b</sup>	4.8 $\pm$ 7.5 <sup>b</sup>
Abnormal cells (%)	ND	ND	88.9 $\pm$ 12.1 <sup>a</sup>	70.6 $\pm$ 23.8 <sup>a</sup>	55.0 $\pm$ 31.8 <sup>ab</sup>	30.4 $\pm$ 25.9 <sup>c</sup>	22.7 $\pm$ 17.1 <sup>c</sup>	26.4 $\pm$ 13.1 <sup>bc</sup>

<sup>a,b</sup> Means within rows with different superscripts differ  $P<0.05$ ; n: number of bulls studied in each age group (in parenthesis); ND: unsuccessful attempt of sperm collection; <sup>†</sup> Scored from 0 (immobile) to 5 (very fast).

Mean body weight, body condition score, and scrotal circumference of G1 and G2 bulls submitted to sexual behavior evaluation were 194.7±37 and 340.3±48.6kg, 2.9±0.4 and 3.7±0.4, and 19.6±2.8 and 29.7±4.0 cm, respectively. Thirty-two percent of G1 bulls and 95% of G2 bulls had reached puberty by the time of sexual behavior evaluation. Moreover, 55 % of G1 and 100 % of G2 bulls had reached puberty by the examination trial subsequent to the sexual behavior evaluation (3 months later). The frequency of bulls exhibiting sexual behavior and the average number of times each behavior was exhibited are shown in Fig. 1. Frequency of bulls smelling and or licking the perineal region of the female, presenting Flehmen

reflex, and approaching and pursuing cows were greater ( $P<0.05$ ) in G2 than G1 bulls for both evaluation methods. The average frequency of each sexual behavior exhibited, regardless of age group, was similar between males evaluated individually or under competition. Twenty-nine percent of the prepubertal males and 88% of the postpubertal males displayed sexual interest for at least one of the two behavioral evaluation methods. The youngest age, at which a prepubertal bull exhibited sexual interest or mounting without penile exposition, was 13.6 or 13.9 months of age, respectively. Only one bull did a single mount with penile intromission (complete mount) during the study.

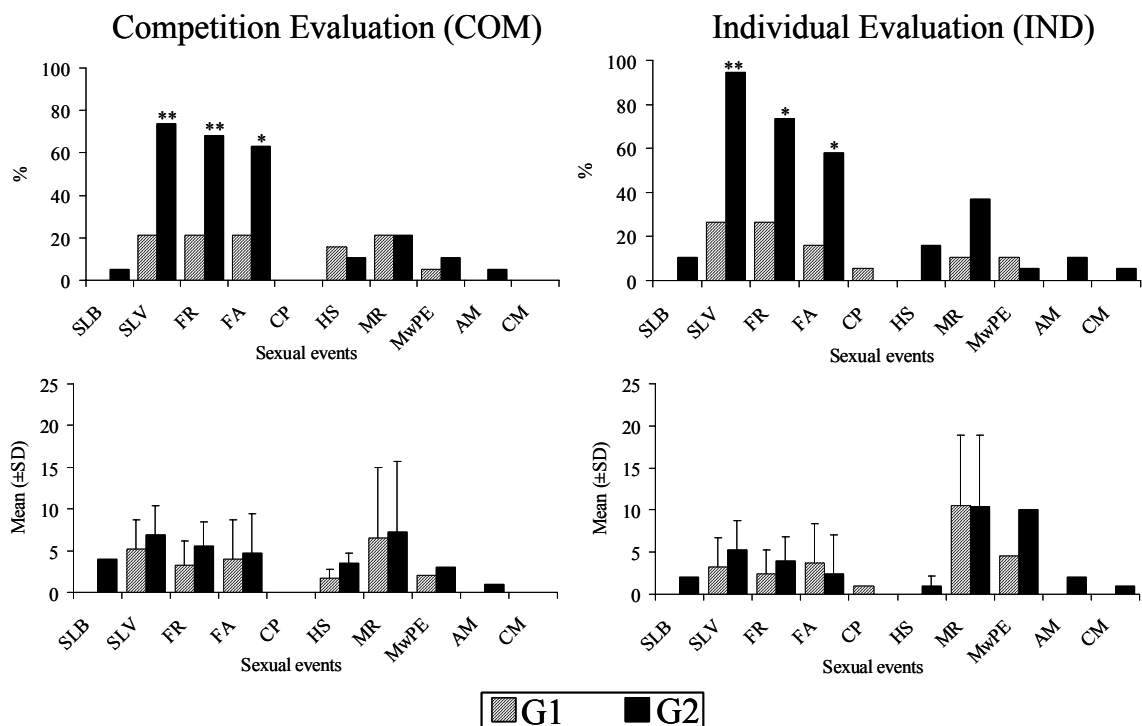


Figure 1. Percentage of sexually inexperienced Guzerat bulls showing sexual behavior and means (±SD) for each of the behaviors in different age groups according to the competition (COM) or the individually (IND) evaluation methods. \*\*  $P<0,01$ ; \*  $P<0,05$ ; duration of each evaluation method = 60 minutes; G1- bulls from 12.1 to 17.4 months old; G2- bulls from 18.4 to 28.4 months old; SLB – smelling and licking cow body; SLP – smelling and licking cow perineal region; FR - Flehmen reflex; FA – female approach and pursuing; CP- chin pressure ; HS - head strikes; MR - mount reflex; MwPE - mount without penile exposition; AM - aborted mount; CM - complete mount.

### Discussion

In the present study, testicular measurements found in the youngest group of males (Table 1) suggest that testicular growth in Guzerat bulls may begin at an earlier age than that studied. Scrotal circumference and testicular diameter had a linear growth rate according to age and body weight within the age range studied. A linear increase in scrotal circumference was also

reported in Brahman (Silva-Mena, 1997), Nelore (Dal-Farra *et al.*, 1998; Gressler, 1998; Ortiz-Peña *et al.*, 2000), Simmental x Nelore (Costa and Freneau, 1999), Hereford, Angus, Brown-Swiss and their crosses, (Lunstra *et al.*, 1978), and Holstein (Jiménez-Severiano, 2002) bulls with an age range from 7 to 23 months of age. A cubic relationship between scrotal circumference and age was described in Gir bulls (Guimarães, 1993), and a quadratic relationship was found in Holstein,





Holstein x Gir, Brown Swiss, Nelore and Guzerat bulls (Freneau, 1991; Trocóniz *et al.*, 1991; Pineda *et al.*, 2000; Salvador, 2001; Jiménez-Severiano, 2002). However, the age range reported in those studies included bulls of up to 50 months of age that were close to or had already reached the testicular growth plateau. According to Quirino *et al.* (1999) and Salvador (2001), testicular growth had reached the plateau after 40 months of age in Nelore bulls. In the group of bulls studied, the average daily increase in scrotal circumference was lower for the oldest group of bulls than for the younger ones; however, testicular growth stabilization had not been reached by 30.7 months of age. Testicular diameter was still increasing at that age while testicular length had stabilized around 22 to 25 months. The evaluation of bulls, older than those used in the present study, is required to determine the age of testicular growth stabilization in the Guzerat breed. Other studies showed similar scrotal circumferences to those found in the present study in similar age groups using a restricted number of Guzerat bulls of higher body weight (Valvasori *et al.*, 1985; Trocóniz *et al.*, 1991; Cartaxo *et al.*, 2001). In those studies, the testicular growth plateau had not been reached by 40 months of age. Nelore bulls handled in field conditions also had similar scrotal circumference measurements (Quirino *et al.*, 1999; Unaniam *et al.*, 2000). However, genetically selected Guzerat, Nelore, Gir, and Brahman bulls, raised under an intensive feeding regime, as well as *Bos taurus taurus* bulls, had higher scrotal circumference measurements at an equivalent age range (Coulter and Keller, 1982; Silva-Mena, 1997; Schmidt-Hebbel *et al.*, 2000a; Jiménez-Severiano, 2002). This suggests that poor nutrition may adversely influence testicular growth. On the other hand, the finding of a great variability in testicular size for a given age of bulls raised on pasture becomes a notable issue for selection programs. Considering that extensive management systems are mostly used in tropical regions, data from bull populations raised within those systems are necessary to establish reference parameters which may be used in selection programs.

It has been reported that *Bos taurus indicus* bulls reach puberty between 10 to 19 months of age, with a scrotal circumference range of 17.5 to 23 cm (Garcia *et al.*, 1987; Vale-Filho *et al.*, 2003; Guimarães, 1993; Godinho, 1970). In the population of Guzerat bulls studied, considering age at the onset of puberty as the age of the first successful semen collection, puberty occurred ranging from 14 to 25 months of age, a wide range. The exact age of the onset of puberty for each bull could not be determined because semen collection attempts were just performed every 3 months. Notwithstanding, an age range of higher incidence of puberty onset could be observed (16 to 18.9 months). It could also be speculated that bulls may not have responded to electro-ejaculation in a given attempt and be included as pubertal in a later age group. To

minimize this, another stimuli session was always done immediately after an unsuccessful attempt. Age at puberty was found to be later in another study using Guzerat bulls Garcia *et al.*, 1987). It has been shown that puberty occurs earlier and that a larger scrotal circumference is found in young *Bos taurus taurus* than in *Bos taurus indicus* bulls (Lunstra *et al.*, 1978; Freneau, 1991; Jiménez-Severiano, 2002).

Twenty centimeters was the threshold scrotal circumference below which sperm cell collection was always unsuccessful. From that size on, successful attempts increased gradually until 31.9 cm. All attempts of sperm cell collection succeeded in bulls having a scrotal circumference beyond that size. Considering that the percentage of successful attempts did not significantly increase in the groups of bulls having scrotal circumference size above 28.9 cm, it may be speculated that sperm production in Guzerat bulls may have been established in most, if not all, males with a scrotal circumference of 29 cm. Unsuccessful attempts of semen collection in bulls with scrotal circumferences greater than 29 cm may be due to an inadequate electro-ejaculation stimulus. For better definition of the variation in scrotal size associated with the beginning of sperm production, semen collection attempts of young Guzerat bulls should be performed on a more frequent basis.

The gradual improvement of seminal traits after puberty has also been reported in previous studies (Guimarães, 1993; Vale-Filho *et al.*, 1997; Schmidt-Hebbel *et al.*, 2000b; Andrade *et al.*, 2001; Salvador, 2001; Cartaxo *et al.*, 2001; Jiménez-Severiano, 2002; Dias *et al.*, 2003; Guimarães *et al.*, 2003). The major sperm morphological abnormality found first in the postpubertal ejaculates was proximal droplets, followed by abnormal heads and tails. Proximal droplets accounted for 67% of the total abnormalities in the first postpubertal ejaculate. Similar results in different breeds have been reported in other studies (Garcia *et al.*, 1987; Freneau, 1991; Guimarães, 1993; Schmidt-Hebbel *et al.*, 2000b). Total abnormalities decreased gradually and reached mean values of about 30 % in the age group of 22 to 25 months. According to these results, the incidence of a higher percentage of semen abnormalities in Guzerat bulls before 22 months of age should not imply that a particular animal has fertility problems because the abnormalities may result from the process of gonadal ripening during postpubertal period (Kasari *et al.*, 1996). However, based on the analysis of the standard deviation, some individuals did not reach normal sperm production until 28 to 30 months of age. The variability in age and scrotal circumference when normal sperm production is achieved deserves further investigation to verify their accuracy in order to use them as predictors of fertility.

Sperm production in the population of Guzerat bulls studied began around 25 months of age when scrotal circumference was about 30 cm. By then, 100 of the bulls had reached puberty and progressive sperm



motility and percentage of normal sperm cells were  $64.8 \pm 24.8$  and  $77.9 \pm 16.0\%$ , respectively, which are acceptable values for natural mating conditions. However, neither in this study nor in others (Garcia *et al.*, 1987; Trocóniz *et al.*, 1991; Cartaxo *et al.*, 2001; Dias *et al.*, 2003), has the age of completion of sexual development been determined. Completion of sexual development depends on the attainment of maximal testicular growth, equilibration of sperm cell production, and complete development of sexual drive. All those reproductive characteristics are important criteria for the selection of bulls for multiple-mating herds on pasture.

The evaluation of sexual behavior performed in the present study indicated that inexperienced Guzerat bulls can exhibit sexual interest before sperm cell production begins. Both of the evaluation methods seemed to be effective in detecting sexual interest of young bulls in both age groups. The observed age effect was more related to the increase in the proportion of bulls showing a given sexual behavior in the older group than to a greater frequency of a given sexual behavior overall. Smelling and licking the perineal region of the cow, Flehmen reflex, female approach and pursuing, and mount reflex, particularly in the individual method of evaluation, were the types of behavior exhibited by a greater number of bulls in the older group. Among the kinds of sexual behavior exhibited, mount reflex was the one most frequently seen and was evident during the individual evaluation method. Previous studies in Zebu bulls suggested that the mount reflex contributed to the identification of females in estrus and the time for mating (Santos, 2001; Costa-e-Silva, 2002; Amâncio, 2003). Santos *et al.* (1999) and Amâncio (2003) reported that mount reflex is the sexual behavior that best correlates with breeding frequency of adult Nelore bulls on pasture among all kinds of sexual behavior studied. Considering those findings, results of the present study may be used to speculate the breeding potential of an individual at an early age.

Jiménez-Severiano (2002) reported that prepubertal Holstein and Brown-Swiss bulls from six months of age and older exhibited sexual interest towards restrained nonestrous females during a 10 min test period in a paddock. It has been reported that bulls of several breeds,  $9.1 \pm 0.3$  months of age, mounted females in heat without ejaculating during a 5 min test period (Lunstra *et al.*, 1978). Hereford bulls exhibited similar behavior at three months of age in group tests of 40 to 120 min. Thus, the exhibition of sexual behavior during the prepubertal period deserves further investigation in *Bos taurus indicus* males. It should be determined whether or not early exhibition of sexual behavior is related to a better breeding potential in adulthood. Other studies suggested that sexual performance data, obtained in serving capacity tests performed in yearling bulls before 18 months of age, may underestimate the mating potential of certain

individuals (Price and Wallach, 1991a), mainly in *Bos taurus indicus* breeds that usually show delayed sexual maturity compared to other breeds (Chenoweth *et al.*, 1996).

The low number of complete mounts recorded between 12.1 and 29.9 months of age suggests that Guzerat bulls raised in the absence of females after weaning have low mating capacity before 24 months of age in time restricted tests although they exhibit active sexual interest. This finding is in agreement with a study conducted with Brahman and Nelore x Brahman (*Bos taurus indicus*) bulls that did not exhibit complete mounts before 20 months of age (Chenoweth *et al.*, 1996). On the other hand, a larger proportion of *Bos taurus taurus* bulls completed mounts than did the bulls observed in the present study that were of a similar age range (Godfrey *et al.*, 1992). Less than 20% of Holstein and Brown-Swiss bulls, 6 to 16 months of age, completed a mount despite a higher proportion of them that exhibited smelling and licking the perineal region of the cow, Flehmen reflex, and an aborted mount (Jiménez-Severiano, 2002). In addition, it was suggested that young bulls would accomplish complete mounts at younger ages if previously located closer to females or submitted to a mating season.

It may be concluded that Guzerat bulls reach puberty between 13 and 25 months of age with a greater incidence between 16 to 19 months. Furthermore, the lower threshold of scrotal circumference, associated to sperm release in the ejaculate, is 20 cm, and full testicular growth may not be reached until 30.7 months of age. Sexual interest in inexperienced young Guzerat males may be expressed before reaching puberty as early as at 13 months of age.

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