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**SCROTAL CIRCUMFERENCE GROWTH AND ITS RELATIONSHIP WITH  
AGE AT PUBERTY IN GUZERAT BULLS**

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*I dedicate this thesis to my parents Edinson and Amalia,  
and to my grandfather Hernan.  
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## RESUMO

Os objetivos deste trabalho foram avaliar em touros Guzerá: seis modelos não lineares para descrever o crescimento do perímetro escrotal; estimar os parâmetros genéticos para o perímetro escrotal, volume testicular e peso, e estimar a correlação genética existente entre estas características com a idade à puberdade; estimar a probabilidade de atingir a puberdade; estimar a correlação entre os parâmetros do modelo não linear com o perímetro escrotal e a idade à puberdade, descrever as variáveis que melhor explicam a variação fenotípica das características avaliadas. No primeiro capítulo foram avaliados os modelos Brody, Logístico, Gompertz, Richards e Tanaka, seus parâmetros foram obtidos pelo procedimento Non Linear Regression (NLIN) do Sistema SAS. Todos os modelos apresentaram valores similares para o  $R^2$ , QME, EPM e DMA. A taxa de crescimento absoluta indicou que o perímetro escrotal cresceu 0,019 cm/dia e atingiu a fase de máximo crescimento, 0,025 cm/dia entre os 318 e 435 dias de idade. O ano, estação de nascimento e fazenda influenciaram os parâmetros A e k do modelo Logístico. No segundo capítulo foram obtidos os componentes de (co)variância pelo método da Inferência Bayesiana. As médias a posteriori da herdabilidade para perímetro escrotal, volume testicular e peso variaram de 0,45 a 0,60, 0,35 a 0,55 e de 0,39 a 0,60, respectivamente. Para a idade à puberdade, variou de 0,46 a 0,55, 0,49 a 0,57 e de 0,49 a 0,62. As correlações genéticas e fenotípicas entre as três características com a idade à puberdade foram negativas e de moderada a alta magnitude. A maior resposta correlacionada com a idade à puberdade foi obtida pela seleção dos machos pelo perímetro escrotal aos 650 dias de idade ( $-119,95 \pm 15,1$  dias por geração). No terceiro capítulo foi estimada a probabilidade do animal ser púbere. O aumento de uma unidade (centímetro) no perímetro escrotal aumentaria em 52% a probabilidade do animal ser púbere, e o aumento em uma unidade na idade (dia) aumentaria em 0,3% a probabilidade de o animal ser púbere. Até 20 cm, 15% dos touros teriam atingido a puberdade, a partir dos 22 cm este percentual se elevou a 30%, e aos 24 cm a probabilidade dos touros atingirem a puberdade alcança 50%. No quarto capítulo foi avaliada a correlação dos parâmetros A e k com a idade à puberdade e o perímetro escrotal aos 650 dias de idade. A maior correlação foi obtida entre a idade à puberdade e o perímetro escrotal (-0,61), e em menor magnitude com os parâmetros k (-0,28) e A (0,18). O primeiro componente principal explicou 51% da variação encontrada nas quatro características. A principal característica encarregada de explicar a variação fenotípica foi o parâmetro k, seguida da idade à puberdade. Assim os resultados deste trabalho indicaram que os parâmetros A e k poderiam ser utilizados para modificar a curva de crescimento do perímetro escrotal; o perímetro escrotal aos 650 dias pode ser utilizado como critério de seleção para precocidade sexual; os animais que atinjam mais rapidamente 24 cm de perímetro escrotal poderão ser considerados como precoces; a taxa de crescimento esta altamente correlacionada com a idade à puberdade e o perímetro aos 650 dias de idade, assim, o perímetro escrotal nessa idade poderá ser utilizado como critério de seleção visando melhoria da precocidade sexual.

Palavras-Chave: curvas de crescimento; Guzerá; modelos não lineares; parâmetros genéticos; puberdade; probabilidade; volume testicular.

## ABSTRACT

The aims of this work were evaluate in Guzerat bulls: six nonlinear models to describe scrotal circumference growth; estimate genetic parameters for scrotal circumference, testicular volume and weight, and the genetic correlation between these traits with age at puberty; estimate the probability of reaching puberty; estimate the correlation between nonlinear parameters with scrotal circumference and age at puberty; describe the variables that better explained the phenotypic variation of this traits. In Chapter 1 were evaluated the Brody, Logistic, Gompertz, Richards and Tanaka models, the parameters were obtained by the Non Linear Regression (NLIN) procedure of the SAS system. All models had similar mean values for  $R^2$ , APE, and MAD. The average growth rate indicated that the scrotal circumference grew from 0.019 cm/day and reached the phase of maximal growth, 0.025 cm/day between 318 and 435 days of age. Year, season of birth, and farm were important source of variation of the A and k parameters of the Logistic model. In Chapter 2 were obtained the (co)variance components using Bayesian methods. Posterior means of heritability ranged from 0.45 to 0.60 for scrotal circumference, 0.35 to 0.55 for testicular volume, 0.39 to 0.60 for body weight. Posterior means of heritability for scrotal circumference, testicular volume and weight ranged from 0.45 to 0.60, 0.35 to 0.55 and 0.39 to 0.60, respectively. For age at puberty using the two-trait analysis with scrotal circumference, testicular volume and weight ranged from 0.46 to 0.55, 0.49 to 0.57, and 0.49 to 0.62, respectively. Genetic correlation between this traits with age at puberty were negatives and from moderate to high magnitude. The most favorable expected correlated response in age at puberty was at 650 d of age ( $-119.95 \pm 15.1$  d per generation). In Chapter 3 was estimated the probability of the animals reaching puberty. The Odds ratio indicated that the increase in one unit of scrotal circumference can increase in 52% the odds of the Guzerat bulls be pubertal, and the increase in one unit of age can increase in 0.3%. Until 20 cm of scrotal circumference, only 15% of Guzerat bulls reached puberty, at 22 cm this percentage increased to 30%, at 24 cm of scrotal circumference the probability was 50%. In Chapter 4 was estimated the correlation between parameters A and k with the age at puberty and scrotal circumference at 650 days of age. The highest correlation was obtained between age at puberty and scrotal circumference (-0.61), and in less magnitude with the k (-0.28) and A (0.18) parameters. The first principal component explained 51% of the variation founded in the four traits. The principal trait that best explain the phenotypic variation was the k parameter, followed by age at puberty. Results obtained in this work indicated that A and k parameters could be used for the modification the scrotal circumference growth curve; scrotal circumference at 650 days of age could be used as criterion selection for sexual precocity; animals that reached most fast 24 cm of scrotal circumference could be considered as precocious; the growth rate is higher correlated with age at puberty and the scrotal circumference at 650 days of age, therefore, scrotal circumference in this age can be used as criterion selection for improvement the sexual precocity.

Keywords: growth curve; Guzerat bulls; nonlinear models; genetic parameters; puberty; probability; testicular volume.

## INTRODUCTION

*Bos indicus* breeds and their crosses with *Bos taurus* predominate in the tropics due to its greater resistance to endo and exo-parasites and heat stress (Turner, 1980). However, animals originating from *Bos indicus* have lower reproductive efficiency (puberty and late sexual maturity) and have lower growth rates than *Bos taurus* (Turner, 1980; Brito et al., 2004; Nogueira, 2004).

The reproductive performance of livestock is one of the main determinants of the beef cattle production efficiency, and should be considered in selection programs (Alencar et al., 1993). However, some reproductive characteristics are limited by sex and some can be measured only in mature individuals. Thus, the ability to identify animals that carry the best allele is severely restricted (Land, 1981).

Reproduction is a complex process and the direct selection of traits of reproductive performance is, at times, difficult to apply (Dias et al., 2008). The identification of easily measured characteristics, that showing genetic variability, and which are genetically correlated with other reproductive characteristics that determine the fertility of bulls, is important to directing genetic improvement programs (Quirino et al., 1999;. Dias et al, 2008).

With increasing demand of semen from high productive and reproductive quality bulls, and with the development of semen freezing techniques, methods to predict the semen production potential and particularly to identify young bulls with high sperm production, were, and are today, object of study. The ability of a bull to produce

semen of good quality and adequate quantity will influence the number of females who may become pregnant, artificial or naturally, and its genetic contribution to subsequent generations (Knights et al., 1984).

The evaluation of sperm quality allows estimating the fertility potential of a bull (Kealey et al., 2006). However, to establish selection strategies and achieve the expected genetic progress, it is necessary prior knowledge of genetic parameters for the interest and the associations between them, and these parameters differ between populations and environments (Barichelo et al., 2010 ).

In the 30's began to be published a series of studies aimed to identifying ways to quantify sperm production capacity of bulls of various breeds. These studies have estimated the number of spermatozooids produced per unit weight of the testes and the relationship between the amount of tissue and testicular sperm production. To that end, the animals were sacrificed to get the actual weight of the testes (Van Demark and Boyd, 1957).

In 1957, Willet and Ohms measured the testes in situ, in the largest point horizontal perimeter of the scrotum, from animals slaughtered. After the in situ measurement, the testes were removed and the testicular and epididymal volumes were calculated. The authors found a high correlation between scrotal circumference and testicular weight (0.94) and between scrotal circumference and sperm production in young bulls (0.96), indicating that the testicular size and sperm production could be estimated accurately by measuring

scrotal circumference. Similar results were reported by other authors (Hahn et al, 1969;. Coulter and Foote, 1976).

The correlation between scrotal circumference and sperm production decreases with the age. This may indicate that the prediction of sperm production and testicular development by the use of scrotal circumference is possible in young animals. However, in older animals, this correlation would not be as efficient, possibly because these animals show a decrease in spermatogenesis (Hahn et al., 1969) or due to the trend found by Coulter and Foote (1977) in which mature and heavy bulls, in which growth has ceased, testes appear to have lower consistency than younger animals. This fact could be associated with the degree of fat deposited in the scrotum.

Favorable genetic correlations of scrotal circumference of young animals (European and Zebu) with parameters indicator of sperm amount and quality, such as semen volume, concentration of the ejaculate, turbulence, motility, percentage of primary and secondary abnormalities, and total percentage of abnormal sperm have been reported by several authors (Bergmann et al., 1997; Silva et al, 2002;. Kealey et al., 2006; Garmyn et al, 2010;. Smith et al, 2011.). These results indicated that selection to increase testicular size can positively influence the fertility of young bulls.

Pacheco et al. (2007) found that the effect of farm influenced the scrotal circumference size and sperm characteristics in Guzerat bulls. Bulls with larger scrotal circumference at 24 months (34.17 versus 29.60 and 27.85 cm) had better sperm quality (motility, vigor and turbulence) and

higher sperm production than animals with smaller scrotal circumference at the same age. These differences are maintained until 48 months of age. However, after 72 months of age, even with a significant difference in scrotal circumference (40.72 and 38.28 cm), there was no significant difference in quality and quantitative semen characteristics of bulls of different farms.

Similarly, Alencar et al. (1993) and Barichelo et al. (2011) reported the influence of environmental factors such as year and season of birth on scrotal circumference of Canchim bulls, indicating that animals weaned on more favorable times, with greater availability of forage, have higher scrotal circumference.

Growth characteristic can be evaluated using non-linear models, which show two parameters with biological interpretation. The parameter A (size of the trait in the adult animal), in this case, is the scrotal circumference that the animal to reach at maturity; and the parameter k representing the maturity index and determines the growth efficiency of the trait measured (Fitzhugh, 1976). The increase in scrotal circumference follows a sigmoid pattern, with a slow initial growth phase, followed by an acceleration phase and an inflection point, where there is a decrease in the growth rate until stabilization of the scrotal circumference size (Quirino et al. 1999).

According to Quirino et al. (1999) and Neves et al. (2011) in Nellore bulls after the inflection point (13 and 11.32 months, respectively), scrotal circumference continues to grow, but suffers a decrease in the growth rate approximately at 30 and 36

months, reaching a plateau between 40 and 48 months approximately.

In Guzerat bulls, Osorio et al. (2012) reported that after the inflection point (13.2 months) the growth of scrotal circumference decreased from 0.58 cm/month to 0.29 cm/month, and up to 36 months of age testes not had stabilized growth.

It has been reported in the literature that environmental factors are responsible for a large variation in the parameters of the growth curves in several species of zootechnical importance. This can occur because of changes in climate and management conditions that affect the quality and availability of pastures (Silva et al., 2001; McManus et al. 2003; Figueireido et al, 2012.). According to Sarmiento et al. (2006), knowledge of the genetic and environmental factors that influence the growth curve, make it possible to change the growth pattern by selecting, or would permit identify animals with higher growth rates but without changing the weight of the adult, instead of selecting increasingly larger animals.

As well as environmental factors influence the parameters of body growth curves of various species, and, as reported earlier, environmental factors influence the scrotal circumference and seminal characteristics of the bulls, possibly those same environmental factors may also influence the parameters of the scrotal circumference growth curves estimated by nonlinear models. However, were not found in the literature reporting about such results.

The early estimate of the parameters of the growth curves of a trait is important to assist

in goal selection, due to the possible correlation between these parameters and other important economic traits (Beltran et al., 1992). According to Taylor and Fitzhugh (1971), early growth animals reach earlier a specific maturity. Beltran et al. (1992) found that cows of slow growth and high weight at maturity also showed low reproductive efficiency when young. Likewise, Barbosa et al. (2002) indicated that depending of the productive efficiency trait that you want to improve, there is a great combination between the rate of maturity and size at maturity. Thus, these results suggest the importance of evaluating the correlation between growths patterns estimated using nonlinear models with traits associated with reproductive efficiency of the animals.

Negative correlation between parameters A and k of nonlinear models has been reported by several authors for various species. This correlation indicates that animals with higher growth rates has less probability to achieve larger sizes (depending on the characteristics evaluated) at adulthood than those who grow more slowly in early life (McManus et al. 2003; Sarmiento et al., 2006; Lamb et al, 2009;. Souza et al, 2010).

Alencar et al. (1993) reported negative genetic correlation (-0.44) between the scrotal circumference at 12 months and the scrotal circumference growth rate between 12 and 18 months, and between 18 and 24 months of age, suggesting that genes that contributing to a larger circumference at 12 and 18 months, act in opposite direction to the scrotal circumference at 18 and 24 months.



We do not find literature about the correlation between the parameters A and k of the scrotal circumference estimated using nonlinear models. However, the negative correlation between these parameters previously reported by various authors for weight, and those described by Brito et al. (2004) and Alencar et al. (2003), could indicate that for the scrotal circumference a negative correlation between these parameters could be found. Thus, the implications when selecting early testicular growth of animals would still need to be elucidated.

Brito et al. (2004) reported that animals who reached puberty earlier had higher scrotal circumference growth rates and higher scrotal circumference at younger ages than later animals. Similar results have been reported by other authors (Lunstra and Echterkamp, 1982). Likewise, animals with delayed testicular development reached maturity older (> 70% normal sperm). These animals, however, had higher scrotal circumference at maturity.

The identification of animals that showing greater testicular size at younger ages than other animals of the same age and raised under the same conditions, would be a tool that could be used in selection programs that

aimed the improving of the sexual precocity in the herds.

Bulls from European breeds reach puberty earlier than zebu animals (Jimenez-Severiano et al, 2002;. Lunstra et al. 2003; Brito et al., 2004); however, between zebu animals there are also differences between breeds and within the same breeds in age at puberty. Assumption et al. (2013) reported high variability in age at puberty in Nellore bulls (between 450 and 540 days of age). Other authors have reported similar results, or even higher ages (Freneau et al., 2006; Brito et al., 2004). For Guzerat bulls have been reported an age at puberty between 547 days (Troconiz et al., 1991; Torres and Henry, 2005) until 622 days (Loaiza-Echeverri et al, 2013.). Because of the variability in age at puberty, the selection of animals by higher scrotal circumference in a specific age for a specific breed may not be suitable for another breed.

The scrotal circumference has been used as selection criteria to improve reproductive efficiency in beef cattle (Baker et al, 1981; Boligon et al, 2007), mainly because it is easy to measure and presented high heritability in *Bos taurus* and *Bos indicus* cattle (Table 1 and 2).

Table 1. Estimates of heritability reported by some authors for scrotal circumference of bulls from *Bos taurus* and synthetic breeds at various ages.

Author	Breed	Age	h <sup>2</sup>
Latimer et al. (1982)	Angus	225 d	0.60
		365 d	0.38
Knight et al. (1984)	Angus	365 d	0.36
		365 d	0.46 e 0.49
Bourdon e Brinks (1986)	Hereford	365 d	
Smith et al. (1989)	Hereford, Angus, Red Angus	365 d	0.40
Nelsen et al. (1986)	Hereford	403 d	0.41
		490 d	0.47
Kriese et al. (1991)	Hereford	365 d	0.53
		365 d	0.16
Alencar et al. (1993)	Canchim	365	0.40
		550	0.36
		24 m	0.31
Silva et al. (2000)	Canchim	365 d	0.30
Martinez-Velázquez et al. (2003)	Red Poll, Hereford, Angus, Limousim, Simmental, Charolais, Pinzgauer, Gelbvieh e Braunvieh	365 d	0.41
		365 d	0.41
Garmyn et al. (2010)	Angus	387 d	0.46

Table 2. Estimates of heritability reported by some authors for scrotal circumference of bulls from *Bos indicus* breeds at various ages.

Author	Breed	Age	h <sup>2</sup>
Vargas et al. (1998)	Brahman	550 d	0.28
Gressler et al. (2000)	Nelore	365 d	0.24
		550 d	0.31
Pereira et al. (2000)	Nelore		0.51
Faria et al. (2004)	Nelore	450 d	0.61
Forni e Albuquerque (2005)	Nelore		0.42 a 0.44
Boligon et al. (2007)	Nelore	365 d	0.25 a 0.26
		550 d	0.35 a 0.37
Faria et al. (2008)	Nelore	365 d	0.49 e 0.60
		365 d	0.29
Frizzas et al. (2009)	Nelore	550 d	0.42
		550 d	0.42
Boligon et al. (2010)	Nelore	270 d	0.29
		365 d	0.39
		550 d	0.42
Boligon et al. (2011)	Nelore	229 d	0.24
		300 d	0.47
		500 d	0.52
Silva et al. (2011)	Nelore	550 d	0.42

Further, scrotal circumference is positively associated with growth (Alencar et al., 1993; Silveira et al., 2004; Castro-Pereira et al, 2007;.. Yokoo et al, 2007) and reproduction characteristics, such as seminal characteristics (Moser et al., 1996; Bergmann et al., 1997; Kastelick et al., 2001; Garmyn et al, 2010;. Smith et al, 2011.) and age at puberty in males (Lunstra and Echterkamp, 1982) and females

(Gressler et al., 2000; Pereira et al., 2000; Boligon et al., 2007).

We do not find literature about genetic parameters of scrotal circumference in Guzarat bulls, even though this is a meet breed commonly used in Brazil. More importantly, was not found in national and international literature, for *Bos taurus* or *Bos indicus* cattle, reported for the

heritability of age at puberty and their genetic correlation with scrotal circumference, testicular volume and weight measured at various ages. Nevertheless, this characteristic is indicated as criterion selection of animals for improvement sexual precocity.

There is a delay in the literature to explain the correlation between scrotal circumference at young ages with scrotal circumference at later ages, and the implication of the selection of animals with early testicular growth and early age to puberty on the reproductive performance of the adult animal.

Because of the facts presented above, the objective of this work was to study the testicular growth, their genetic parameters and their relationship with age at puberty and body growth in Guzarat cattle, to support its use in the selection programs for this breed.

### **SPECIFIC OBJECTIVES**

- Choose the nonlinear model which the best fits to the scrotal circumference data and describe the average growth curve of the scrotal circumference in Guzarat bulls using nonlinear models;
- Identify the influence of environmental factors (farm, year and season of birth) on the parameters A and k of scrotal circumference growth curve of Guzarat bulls;
- Estimate genetic parameters for scrotal circumference, testicular volume, body weight (measured at different ages) and age at puberty, determine its correlation and estimate the correlated response in age at

puberty for the selection of scrotal circumference, testicular volume and weight;

- Estimate, using the logistic regression, the probability of Guzarat bulls reaches puberty due to the scrotal circumference size and age;
- Evaluate a set of characteristics (A and k parameters estimated by nonlinear model, scrotal circumference at 650 days of age and age at puberty), through principal component analysis, to identify which of these traits representing most of the phenotypic variance in Guzarat bulls;
- Estimate the correlation between the characteristics evaluated in the principal component analysis.

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## **GENERAL DESCRIPTION OF THE MATERIAL**

The data used in this work are from Guzerat bulls bred in extensive grazing conditions on three farms located in the State of Minas Gerais, and born between the years 2000 and 2012. The climatic conditions and the similar management of three farms was one of the starting points for their choice, like the availability of staff for help during data collection, which implied the movement of a large number of animals per day and management in the corral, and the interest of

the owners of the farms to help with the development of the research, permitting the handling of the animals and the frequent visits to their properties, and availability of access to the data of each one of the farms (data consisted of the genealogy of the animals, dates of birth, weighing, phenotypic data from parents, etc).

### **Farms**

The farms are located in the municipalities of Brasilândia, Unai and Carlos Chagas in the State of Minas Gerais. The municipality of Carlos Chagas has the geographical coordinates 17°41'30" south latitude and 40°45'15" west longitude and altitude of 159 meters above sea level. The region has a maximum temperature of 30°C and a lowest of 18°C and average annual rainfall of 1,059 mm. Unai has the geographical coordinates of latitude 16°21'43" and 46°54'09" west longitude and altitude of 600 meters over the sea level. The region has a maximum temperature of 35°C and a lowest of 24°C and average annual rainfall of 1200 mm; and the municipality of Brasilândia of Mines, has as geographic coordinates 17°00'36" south latitude and 46°00'32" west longitude and altitude of 520 meters over the sea level. The region has an average temperature of 22.6°C. The farms are located in two regions of the State of Minas Gerais, two of which are in the Northwest region (Brasilândia and Unai) and the third farm in the northeast region state's (Carlos Chagas). The climate classification of Köppen climate for these regions is tropical type AW, or tropical savannas, characterized by two seasons: dry (May to October) and rainy (November to April).



## **Herd and management**

### *Guzerá do Rosario Farm (Carlos Chagas)*

This farm weaning is done twice during the year. Calves born between September and December and are separated from their mothers before the start of the next breeding season, which begins in July and runs until August. Calves born between April and June are separated from their mothers before the start of the breeding season in December. In both cases there may be variations in the dates of weaning, according to the rainfall and pasture condition of the farm. In years when the dry season is very strict, weaned calves are supplemented with sugar cane and urea for some few months until weather conditions improve.

During the growing period, the animals remain in *Brachiaria brizantha* pastures and are supplemented with mineral salt without urea; between May and June mineral supplementation is the protein and type. The bulls that are selected for sale are supplemented with sugar cane and urea during the dry season. In the health management, all animals are vaccinated counter various diseases.

### *Reunidas Antônio Balbino Farm (Brasilândia de Minas)*

In this property births occur between the months of September to December. The calves, after birth are kept with their mothers in pastures formed by grass *Andropogon gayanus*, receiving mineral supplementation and diet in creep feeding system until weaning, which is made when the animals reach seven months of age.

All animals under 21 months of age are routinely heavy for the evaluating of their

body development. During the rearing, in the rainy season, the animals remain in pastures of *Brachiaria brizantha* and *Andropogon gayanus*, and supplemented with mineral salt without urea. Between May and June mineral supplementation is the protein type. In the months of July, August, September and October the animals are confined and subjected to balanced diet of sorghum silage and solid feed. Starting at 24 months, bulls entering the breeding season to the common cows, after which they are sold. In May, all animals are vaccinated.

### *Palestina Farm (Unai)*

In this farm breeding season begins on January 1<sup>o</sup> and ends on March 30, with birth calves taking place from October until December. The calves are weaned at seven months of age. During the growing period males remain in pasture with a predominance of *Panicum maximum* grass. During the rainy season the animals do not receive protein or energy supplementation. Already, during the dry season, are supplemented with protein salt and maintained in pasture with *Brachiaria Brizantha*. The mineral salt is provided throughout the year. This farm participates in breeding programs of the ABCZ (Brazilian Association of Zebu Breeders) and EMBRAPA, where the bulls are weighing every three months, in addition to weighing made at birth and at weaning. All farm animals are vaccinated.

## CHAPTER 1

### USE OF NONLINEAR MODELS FOR DESCRIBING SCROTAL CIRCUMFERENCE GROWTH IN GUZERAT BULLS RAISED UNDER GRAZING CONDITIONS

#### Abstract

Different nonlinear models to describe scrotal circumference (SC) growth in Guzerat bulls were compared. This study was conducted on three farms in the state of Minas Gerais, Brazil. The nonlinear models were: Brody, Logistic, Gompertz, Richards, Von Bertalanffy and Tanaka, where parameter A is the estimated testis size at maturity, B is the integration constant, k is a maturing index and, for the Richards and Tanaka models, m determines the inflection point. In Tanaka, A is an indefinite size of the testis, B e k adjust the shape and inclination of the curve. A total of 7410 SC records were obtained every 3 months from 1034 bulls with ages varying between 2 to 69 months (<240 d of age = 159; 241 to 365 d = 451; 366 to 550 d = 1443; 551 to 730 d = 1705 and >731 d = 3652 SC measurements). Goodness of fit of the models was evaluated by the coefficients of determination ( $R^2$ ), error sum of squares (ESS), average prediction error (APE) and mean absolute deviation (MAD). The Richards model did not reach the convergence criterion. The  $R^2$  were similar for all models, with values between 0.68 and 0.69. The ESS was lowest for the Tanaka model. All models fit the SC data poorly in the early and late periods. Logistic was the model which best estimated SC in the early phase as indicated by the APE and MAD. The Tanaka and Logistic models had the lowest APE between 300 and 1600 d of age. The Logistic model was chosen for analysis of the environmental influence on parameters A and k. The absolute growth rate (AGR) indicated that SC growth increased 0.019 cm/d reaching the maximum value of 0.025 cm/d between 318 and 435 d of age. Farm, year and season of birth affected significantly size of adult SC and growth rate. An increase in SC adult size (parameter A) was accompanied by the decrease of the SC growth rate (parameter k). In conclusion, SC growth in Guzerat bulls was characterized by an accelerated growth phase, followed by decreased growth, this was best represented by the Logistic model. The inflection point occurred around 376 d of age (mean SC of 17.9 cm). We inferred that early selection by testicular size may result in smaller testicular size at maturity.

*Key words:* Environmental factors; Growth curve; Guzerat bulls; Nonlinear models; Scrotal circumference

#### Introduction

Scrotal circumference (SC) is frequently used in breeding programs, because of its easy measurement, high repeatability, and moderate to high heritability (Brinks et al., 1994; Gressler et al., 2000; Lunstra et al. 1978; Oliveira et al., 2007). In addition, SC

is favorably associated with physical semen characteristics, age at puberty, sexual precocity, and weight gain (Bergmann et al., 1997; Brito et al., 2004; Silva et al., 2004; Smith et al., 1989). Furthermore, based on the genetic correlation between SC and reproductive characteristics in females, e.g., age at puberty, days to calving, and age at

first calving, selection for SC had a positive influence on female reproductive performance (Forni et al., 2005; Moser et al., 1996; Smith et al., 1989; Vargas et al., 1998).

Zebu cattle are older at puberty and have delayed first calving (30–46 months) (Dias et al., 2004; Perotto et al., 2001); these are attributed to genetic and environmental factors (Nogueira et al., 2004). Bulls selected on the basis of larger SC and early puberty usually produce daughters reaching puberty at significantly earlier ages and greater percentages of heifers cycling early in the breeding season, even when used in crossbreeding (Moser et al., 1996; Smith et al., 1989). This is very important for cattle breeding in tropical countries, where delayed puberty and poor reproductive efficiency are major constraints limiting the cattle industry (Syrstad et al., 1998).

The SC at puberty is relatively constant among breeds and across bulls varying widely in age and weight at puberty; therefore, SC might be more useful than other characteristics for predicting age at puberty (Bourdon et al., 1986; Lunstra et al., 1978; Siddiqui et al., 2008). Scrotal circumference is more easily obtained than sperm production or behavioral measurements and should be useful in the selection of beef bulls for early sexual maturity (Lunstra et al., 1978).

Selection of bulls at the earliest possible age will not only improve reproduction, but also provide economic advantages by decreasing feeding and management costs and improve economic returns to producers (Barth and Ominski, 2000). However, Lunstra et al. (1978) reported that male offspring of

diverse heat-adapted sire breeds had lower postweaning testicular growth rates and were slower to reach puberty than offspring of European sire breeds. Therefore, there is a need for characterization of reproductive traits in bulls representing diverse beef breeds.

Furthermore, nutrition, breed, and environmental factors influence reproductive development and testicular size (Coulter et al., 1976; Tatman et al., 2004). Therefore, to achieve higher accuracy in assessing the reproductive capacity of bulls based on testicular measurements, comparisons should be made within the same age group, breed, bull stud, and year-season (Santoro et al., 2005).

One way to describe testicular growth is using nonlinear regression models. The advantage of nonlinear models is that they can accommodate a large number of measurements in some parameters and, thus, permit appropriate biological interpretation. The benefit of these models in animal breeding is using these parameters to identify animals more suited for specific purposes (Lunstra et al., 2003). Furthermore, a growth trait can be improved by using growth curves and their properties. In that regard, Kratochvílová, et al. (2002) identified early and late maturing heifers during early phases of growth (6–9 months) using growth curve parameters of nonlinear models.

Given the growing interest in Zebu cattle because of their better adaptation than *Bos taurus* breeds to tropical conditions, and the need for producers to emphasize growth rate and early pubertal development in genetic improvement programs (Siddiqui et al.,

2008), the objective of this study was to evaluate six nonlinear models to describe SC development in Guzerat bulls and to evaluate environmental effects on growth curve parameters.

## **Material and methods**

### ***Animals and scrotal circumference measurement***

All procedures performed in this study were approved by the ethics committee of Universidade Federal de Minas Gerais.

This study was conducted on three farms located in Brasilândia de Minas (17° 00' 36" South and 46° 00' 32" West), Carlos Chagas (17°41'30" South and 40°45'15" West), and Unaí (16°21'43" South and 46°54'09" West), in the state of Minas Gerais, Brazil.

The climate classification in this regions is Aw (tropical rainy climate, Köppen classification), with average temperature of 18° in the coldest month. The dry season coincides with fall and winter, principally, and these seasons go from April to September. Spring and summer go from October to March. November, December, January and February concentrated higher rainfall, approximately 60% of the total yearly rainfall (Sá Junior, 2009).

During the nursing period and after weaning (~ 7 months old), all Guzerat males were raised under grazing conditions on palisade grass (*Brachiaria brizantha* Stapf) and bluestem (*Andropogon gayanus* Kunth) in the savannah region with water and mineral

salt *ad libitum*. During the dry season the animals were supplemented with salt-type protein or roughage.

Scrotal circumference was measured in the region of the greatest diameter of the testes and covered two gonads positioned symmetrically side by side, leaving the skin of the scrotum distended. Measurements were performed every 3 months, most of them beginning around 240 d of age (SC measurements: < 240 d = 159; 241 to 365 d = 451; 366 to 550 d = 1,443; 551 to 730 d = 1,705 and >731 d = 3,652), and continuing throughout the period the animal remained on the farms.

Before analysis, the initial database was edited to enable the environmental factor study on the three farms. Therefore, a final database of 7410 SC measurements of 1034 males, born between 2001 and 2007, aged between 2 to 69 months, with SC from 11 to 44 cm were used in the two stages of this work.

### ***Statistical analyses***

#### ***Nonlinear models***

Estimates of SC growth curves were obtained based on all SC-age data, using five nonlinear asymptotic models and one nonlinear indeterminate model (Table 1). The asymptotic models describe a growth that never exceeds a horizontal asymptote to infinity ( $SC(t) = \infty SC$ ), whereas the Tanaka model allows indeterminate growth without an asymptote (Bilgin et al., 2004).

Table 1. Nonlinear models evaluated in this study to describe scrotal circumference (SC) growth in Guzerat bulls.

Model	Equation
Brody	$SC_t = A[1 - B \exp(-kt)]$
Logistic	$SC_t = A/[1 + B \exp(-kt)]$
Gompertz	$SC_t = A \exp[-B \exp(-kt)]$
Richards	$SC_t = A[1 + B \exp(-kt)^m]$
Von Bertalanffy	$SC_t = A[1 - B \exp(-kt)]^2$
Tanaka	$SC_t = (1/\sqrt{B}) \ln \left  2B(t - m) + 2\sqrt{B^2(t - m)^2 + AB} \right  + k$

SC<sub>t</sub> = scrotal circumference to t days of age; A = scrotal circumference at maturity; B = proportion of the asymptotic mature testis size to be obtained after birth; k = maturing index; m = inflection point.

In the nonlinear models used to model SC-age relationship, SC<sub>t</sub> is the scrotal circumference (SC) to t days of age, A is the estimated SC at maturity, B indicates the proportion of the asymptotic mature testis size to be obtained after birth, established by the initial value of SC and t; k is a maturing index, establishing the earliness with which SC approaches A. Parameter m shapes the growth curve and therefore determines its inflection point, which indicates the time at which growth acceleration ends and the self-inhibition phase starts until reaching SC size at maturity (Quirino et al., 1999; Notter et al., 1985). In the Tanaka model, A is the indeterminate testis size, B and k adjust the shape and slope of the curve and m is the abscissa of the inflection point. It is noteworthy that the latter is the only parameter with biological interpretation in this model (Bilgin et al., 2004).

The inflection point for SC and age at the inflection point were calculated using the following formulas: for the Logistic model  $\hat{A}/2$  and  $\hat{t} = \ln(\hat{B})/\hat{k}$  (Notter et al., 1985); for the Gompertz model,  $\hat{A} * 0.368$

and  $\hat{t} = \ln[\ln(\hat{A} * 0.37)/(-\hat{B})]/-\hat{k}$  (Nieto et al., 2006); and for the Von Bertalanffy model,  $\hat{A} * 0.2963$  and  $\hat{t} = \ln[(1 - (\hat{A} * 0.3)^{\hat{B}})/(-\hat{k})]$  (Nieto et al., 2006). The Brody model was originally intended to describe growth occurring after inflection, and, therefore, this model has no inflection point (Beltran et al., 1992). In the Richards and Tanaka models the inflection point is represented by parameter m (Nieto et al., 2006).

The Gauss-Newton iterative method from Non Linear Regression (NLIN) of the SAS System (SAS Institute, 2001) was used to estimate the parameters for each nonlinear model.

### Goodness of fit

Goodness of fit of models were evaluated according to the following criteria: (1) The R<sup>2</sup> which determines the percentage of variation in Y, explained by the statistical model (Neter J, and Wasserman, 1974). This value was derived from the square of the sample correlation coefficient between the

observations and their predicted values; (2) Error sum of squares (ESS) was considered as an accepted control and quality measurement and was calculated as follows:  $ESS = \sum(Y_i - \hat{Y}_i)^2/n$ , where the deviation of an observation  $Y_i$  is calculated around its own estimated mean  $\hat{Y}_i$  (Neter J, and Wasserman, 1974; Posada and Noguera, 2007); (3) The average prediction error (APE) that quantifies the relative disagreement between observed and predicted SC values for each specific age which was calculated for each model, as follows:

$APE\% = [(SC - PSC)/SC] * 100$ , where SC and PSC are the mean of the observed and predicted SC, respectively, for each specific age (Goonewardene et al., 1981; Mazzini et al., 2003); (4) mean absolute deviation of the residuals (MAD) which was compared among models and estimated by:  $DMA = \sum_{i=1}^n |Y_i - \hat{Y}_i|/n$ , where  $Y_i$  is the SC observed,  $\hat{Y}_i$  is the SC estimated by the model and n is the total of measurements (Sarmiento et al., 2006). The ESS, APE and MAD were calculated grouping the ages (in days) on a monthly basis.

After selecting of the best model, the absolute growth rate (AGR) for SC was calculated by the following formula:

$\hat{Y}_t \hat{B} \hat{k} / (1 + \hat{B} \hat{C}_1) \hat{C}_1$  where  $\hat{C}_1 = \exp(-\hat{k}t)$ . In this formula,  $\hat{Y}_t$  is the SC estimated by the model in t age,  $\hat{B}$  and  $\hat{k}$  are the parameters estimated by the model chosen. The absolute growth rate represents the size gained per time unit (Malhado et al., 2009).

### *Effects of environmental factors on parameters A and k of the model chosen*

We evaluated the effects of farm (1, 2, and 3), year (2001–2007), and birth season (December 21 to March 20 ¼ summer; March 21 to June 20 ¼ autumn; June 21 to September 20 ¼ winter; and September 21 to December 20 ¼ spring) on parameters A and k of the model chosen in the first stage of the study. On farm 1, the effects of year (2001–2004) and birth season (fall, spring, and summer) and their combinations were evaluated. On this farm, the quantity of animals born in winter in the same years was very low; therefore, season was not analyzed. However, on farms 2 and 3, the effects of farm, year (2006–2007), birth season, and their combinations were analyzed. In this case, only the summer of 2006 was not evaluated, because on the two farms, SC measurements began after the animals born in this season were more than 2 years old. The environmental effects were tested according to the test for parameter equality in nonlinear regression models (Regazzi, 2003; Regazzi, 2004). This test, based on F statistics for lack-of-fit, was obtained after adjusting a reduced model ( $H_0: A_1 = A_2$ ) and a complete model ( $H_a$ : not  $H_0$ ).

### **Results**

The Richards model did not reach the convergence criterion nor did it produce biologically interpretable parameters. For the Brody, Gompertz, Logistic, Von Bertalanffy, and Tanaka models, the values of the parameters estimated and standard error are shown (Table 2). Regarding parameter estimates, the Gompertz and Von Bertalanffy models had close values for SC at maturity (parameters A), and the Brody

model estimated the highest value for A and the lowest value for the k parameter compared with the other asymptotic models. The Logistic model estimated the lowest

predicted SC at maturity (parameter A),  $35.9 \pm 0.26$  cm, and the highest values for B and k (Table 2) between all models.

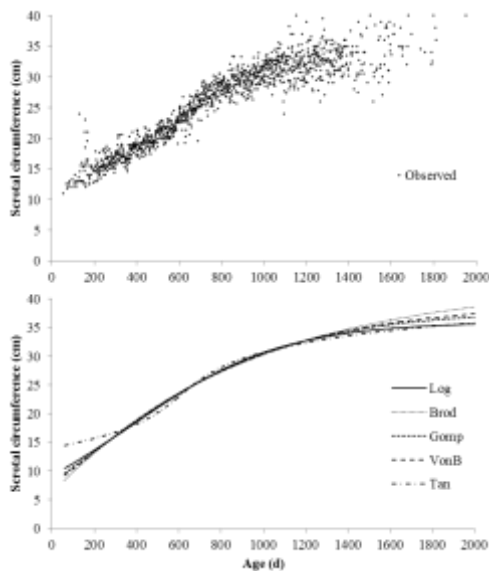
Table 2. Parameters estimates ( $\pm$  SEM), coefficient of determination ( $R^2$ ), error sum of squares (ESS), average prediction error (APE) and mean absolute deviation (MAD) for five models used to describe scrotal circumference growth in Guzerat bulls.

Models	$\hat{A}^1$	$\hat{B}^2$	$\hat{k}^3$	$\hat{m}^4$	$R^2$	ESS	APE	MAD
Brody	42.94 $\pm$ 0.7	0.85 $\pm$ 0.006	0.001 $\pm$ 0.006		0.67	12.3	-1.99	2.78
Logistic	35.96 $\pm$ 0.2	2.86 $\pm$ 0.05	0.002 $\pm$ 0.00005		0.68	12.0	-1.87	2.73
Gompertz	38.06 $\pm$ 0.3	1.54 $\pm$ 0.01	0.001 $\pm$ 0.00005		0.68	12.1	-1.91	2.75
Von Bertalanffy	39.19 $\pm$ 0.4	0.42 $\pm$ 0.004	0.001 $\pm$ 0.001		0.67	12.2	-1.93	2.76
Tanaka	1048.5 $\pm$ 55.7	0.05 $\pm$ 0.004	11.91 $\pm$ 0.44	610.3 $\pm$ 6.2	0.69	11.7	-1.94	2.70

$^1\hat{A}$  = scrotal circumference at maturity;  $^2\hat{B}$  = integration constant;  $^3\hat{k}$  = rate of maturity;  $^4\hat{m}$  = inflection point.

Logistic, Gompertz, Von Bertalanffy, and Brody models depicted similar growth curves approximately between 220 and 1150 days of age. The Tanaka model had the most sigmoid curve (Figure 1) and estimated the highest values for early ages.

Figure 1. Scrotal circumference (SC) observed and estimated by five nonlinear models in Guzerat bulls.



The Brody model estimated the lowest values of SC at early age, and the highest SC in the later periods. The  $R^2$  was similar among models (range, 0.68–0.69). All models had similar mean values for ESS, APE, and MAD (Table 2). Based on the ESS, the Tanaka model had the lowest sum of square, followed by the Logistic model (Table 2). Average prediction errors associated with prediction of SC at each age are shown (Figure 2). For all models, differences between predicted and actual values alternated in sign (positive and negative). Unlike the other models, the Tanaka model overpredicted SC at early ages. The Logistic, Gompertz, Von Bertalanffy, and Brody models had a similar pattern of APE from approximately 200 to 1500 days. From 60 to 122 days of age, the Logistic model had the lowest APE ( $\pm 8\%$ ). From 274 to 1677 days of age, the Tanaka and Logistic models alternated the lowest

SC prediction errors, whereas, at the oldest ages, the Von Bertalanffy and Gompertz models had the lowest prediction errors.

Among the Logistic, Gompertz, Von Bertalanffy, and Brody models, the Logistic model had the lowest APE.

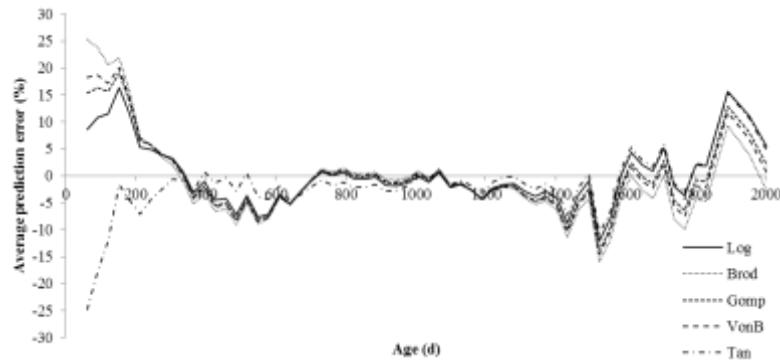


Figure 2. Distribution of average prediction error defined by five nonlinear models to scrotal circumference data in Guzerat bulls. Brod, Brody; Gomp, Gompertz; Log, Logistic; Tan, Tanaka; Von, Von Bertalanffy.

The inflection points for SC and age at inflection point estimated in this study differed among models. For the Von Bertalanffy, the inflection point of SC growth was estimated at 11.6 cm and 4.8 months; for the Gompertz model it was 14 cm and 7.5 months of age; and for the Logistic and Tanaka models, estimates were 17.9 cm at 12.5 months and 23.2 cm at 20.3 months of age, respectively.

Based on MAD (Figure 3), small differences among models were apparent. The smallest deviation was presented by the Logistic models from early age to approximately 1600 days, followed by the Tanaka model. The Brody model had the lowest deviations at late ages among all models evaluated.

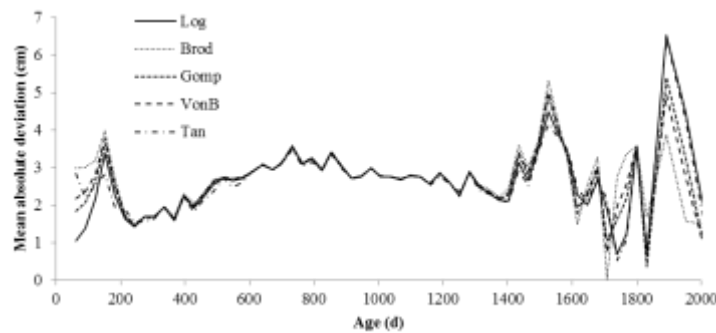


Figure 3. Distributed of mean absolute deviation defined by five nonlinear models to scrotal circumference data in Guzerat bulls. Brod, Brody; Gomp, Gompertz; Log, Logistic; Tan, Tanaka; Von, Von Bertalanffy.



Based on goodness of fit, Logistic was the model with the lowest prediction errors and the smallest deviations from early ages; therefore, this model was regarded as having the best fit to the SC data in Guzerat bulls. The Tanaka model also had good fit to the SC data, but in this model the only parameter having a significant biological interpretation is “m,” which corresponds to the age of the inflection point, and the Logistic Model allowed a simultaneous interpretation of several parameters. Therefore, environment effects on the parameters and the AGR were studied through the Logistic model.

The AGR based on the first derivative of Logistic model in relation to time is shown (Figure 4). Values for AGR increased from 0.019 cm/d and reached a maximum value of 0.025 cm/d between 318 and 435 days of age.

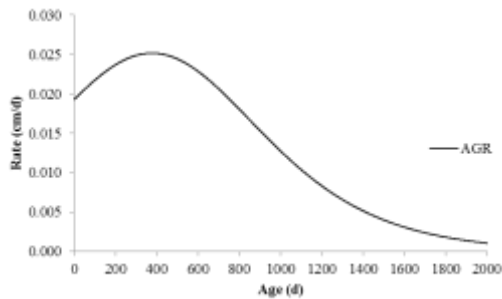


Figure 4. Absolute growth rate (AGR) of the scrotal circumference (SC) in Guzerat bulls based in the Logistic model.

Year, season of birth, and farm were important source of variation of the A ( $P < 0.001$ ) and k ( $P < 0.001$ ) parameters in the three farms. In farm 1, bulls born in 2001 had the smallest A ( $P < 0.001$ ) and the largest k ( $P < 0.001$ ) values, with a gradual increase ( $P < 0.001$ ) in the adult testis size from years 2001 to 2004. Additionally, k

parameter values decreased ( $P < 0.001$ ) during this interval (Figure 5).

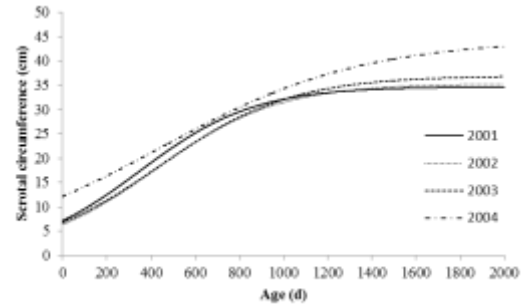


Figure 5. Effect of year of birth on scrotal circumference growth curve in Guzerat bulls in farm 1.

In farm 2, there was an increase in adult testis size ( $P < 0.001$ ) and a decrease in k parameter values ( $P < 0.001$ ) from years 2006 to 2007 in spring-born bulls, whereas the opposite was observed in winter-born bulls (Figure 6). In farm 3, there was a decrease in adult testis size ( $P < 0.001$ ) and an increase in k parameter ( $P < 0.001$ ) from years 2006 to 2007 in spring-born bulls, with the opposite for winter-born bulls (Figure 6).

In farm 2 (2006) and farm 3 (2007) winter-born bulls had SC at maturity greater than spring-born bulls ( $P < 0.001$ ), but the latter had a greater testis growth rate ( $P < 0.001$ ) and reached testis size at maturity faster, although mature testis size was lower (Figure 6).

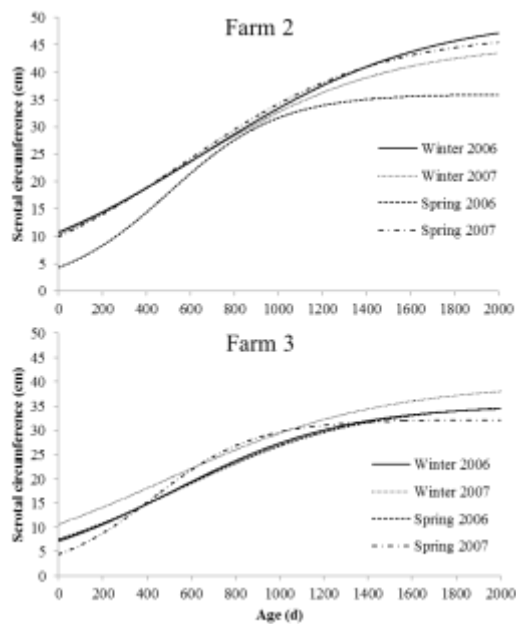


Figure 6. Effect of season of birth, year and farm on scrotal circumference growth curve in Guzerat bulls.

## Discussion

The nonconvergence of the Richards model found in this study was also reported by others using this model to assess SC growth in sheep (Bilgin et al., 2004), goats (Sousa et al., 1997), and cattle (Quirino et al., 1999). These results contrasted with those reported by Delgado et al. (Delgado et al., 2000) and Jiménez-Severiano et al. (2010) where the Richards model yielded good fit to the data for SC in Retinto bulls and Blackbelly ram lambs, respectively. Several authors warned about the difficulty of convergence of this model, because parameter  $m$  (the inflection point) is variable, which can make it more difficult to obtain estimates (Brown et al., 1976; Freneau et al., 1997; Delgado et al., 2000; Silva et al., 2001; Carneiro et al., 2009; Jiménez-Severiano et al., 2010). Furthermore, the high negative correlation between  $m$  and  $B$  (Brown et al., 1976), and

the lack of data at critical parts of the curve (e.g., around the inflection point) (Richards, 1959), are other sources of difficulty in the iterative procedure. In the present study, perhaps the lack of data at early age was a major limitation of this model.

Parameter  $A$  is interpreted as testis size at maturity. Several studies have reported overestimation of SC at maturity by the Brody model in bulls (Freneau et al., 1997; Quirina et al., 1999; Delgado et al., 2000; Nieto et al., 2003; Nieto et al., 2006; Parma et al., 2006), ram lambs (Jiménez-Severiano et al., 2010), and goats (Sousa et al., 1997). The same was found in this study, indicating little use of this model in estimated SC at maturity in Guzerat bulls.

Parameter  $B$  relates SC growth from birth to maturity; therefore, high values of  $B$  represented slow testicular development (Nieto et al., 2006). The values estimated for parameter  $B$  for Guzerat bulls in this study were lower than those reported for Nellore bulls (Freneau et al., 1997; Quirino et al., 1999; Parma et al., 2006; Neves et al., 2011) with the Logistic model. However, results for Guzerat bulls were similar to those obtained in Canchim bulls (Nieto et al., 2003; Nieto et al., 2006). According to Nieto et al. (Nieto et al., 2006), this might be an indicator of greater testicular development of Canchim bulls, or, in this case, Guzerat bulls, in relation to the breeds mentioned above.

Parameter  $k$  is related to growth rate and determines the slope of the curve (Forni et al., 2009). The value of  $k$  obtained in this study by the Logistic model seemed less than values reported by others in Retinto (Delgado et al., 2000), Canchim (Nieto et al., 2006; Nieto et al., 2003), and Nellore

bulls (Freneau et al., 1997; Quirino et al., 1999; Parma et al., 2006; Neves, 2011). Perhaps some heat-adapted sire breeds had delayed testicular development compared with sire breeds not adapted to heat (Lunstra and Cundiff, 2003). Conversely, some breeds that originated from India have accelerated testis growth or constant testicular growth rate after puberty and earlier maturation (Brito et al., 2004; Freneau et al., 2006).

Early maturation is a desirable feature in beef cattle production; it hastens the onset of sexual maturity, which can decrease production costs, reduce generation intervals, and increase genetic gains and overall productivity (Brito et al., 2004). Early-maturing bulls are younger than late-maturing bulls at puberty (Lunstra and Echternkamp, 1982; Brito et al., 2004) and attain puberty during the phase of rapid SC growth, whereas late-maturing bulls attain puberty immediately before entering a plateau phase of SC development (Brito et al., 2004).

All models had poor fit during the early and late phases of SC growth. Other workers reported a good fit for several nonlinear models in late periods (Quirino et al., 1999; Jiménez-Severiano et al., 2010). The Brody model underpredicted SC at early ages and overpredicted adult SC, in agreement with other reports (Freneau et al., 1997; Quirino et al., 1999; Nieto et al., 2003; Nieto et al., 2006; Parma et al., 2006; Jiménez-Severiano et al., 2010), and could be attributed to the reliance of Brody's curve on input data (DeNise and Brinks, 1985).

The Tanaka model, which had a more sigmoid curve, overpredicted SC at early ages. These results were in agreement with

previous reports in that the models fit poorly actual data when expected to project beyond the range of information (e.g., when birth and mature weights were absent (DeNise and Brinks, 1985). Similar to previous reports (Quirino et al., 1999), the Logistic model had a small APE from early ages to more than 900 days of age, and provided relatively better fit than the Gompertz, Von Bertalanffy, and Brody models.

Based on the  $R^2$ , all models were adjusted similarly to SC data of Guzerat bulls; however, values seemed low compared with other studies which used nonlinear models to describe SC growth in bulls (Quirino et al., 1999; Delgado et al., 2000). Perhaps this was because of data being derived from three farms located in different regions, with some differences in management, nutrition, and environmental characteristics. It was noteworthy that Sarmiento et al. (Sarmiento et al., 2006) concluded that evaluating quality of fit in nonlinear models based only on the coefficient of determination might not be a good option.

The ESS indicated that, regardless of small differences between models, there was greater variation in the Brody model, followed by the Von Bertalanffy and Gompertz models. These results were in agreement with previous studies of SC in Nellore and Canchim cattle (Quirino et al., 1999; Nieto et al., 2006).

The inflection point would be associated with a period of rapid proliferation of testicular parenchyma, which suggests the onset of puberty (Quirino et al., 1999). The Brody model has no inflection point; therefore, it provides no definition of increasing and decreasing acceleration phases of sigmoid growth (Beltran et al.,

1992). Brody (1945) suggested using this curve for more than 30% mature animals, or after inflection, or as he termed it, during “the self-inhibiting phase of growth”.

Although Brody suggested that his equation be used to describe growth occurring only in the interval after inflection, he did not restrict it by defining an initial value (Brown et al., 1976).

When used to describe body weight development in Angus cattle, growth curves fitted with the Brody model with data including preinflection points, overestimated birth weight, and underestimated weaning weights (Beltran et al., 1992). Furthermore, in Dorper sheep crosses, this model underestimated growth at initial stages, and it overestimated it from 50 to approximately 140 days of age. This problem can be ameliorated by restricting analysis to data sets that include only “postinflection” periods (Doren et al., 1989).

In this work, the inflection points calculated by the various models were different. The Von Bertalanffy and Gompertz models estimated the inflection point at very low age, near the weaning age established on the three farms studied. Valvasori et al. (Valvasori et al., 1985) reported that Guzerat bulls had an increased SC growth rate from 12 to 14 months and that this period coincided with puberty. In the present study, the Logistic model estimated the inflection point at 12.5 months of age. Troconiz et al. (1991) obtained different results to those reported previously, where the highest rate of testicular growth in Guzerat bulls occurred between 22 and 24 months of age with  $29.1 \pm 2.3$  cm of SC. In the same study, puberty was reached at  $18.2 \pm 0.2$  months of age and  $25.6 \pm 0.3$  cm of SC, values similar

to previous reports for this breed (Garcia et al., 1987; Torres-Júnior and Henry, 2005).

A parallel study carried out in part of the population of Guzerat bulls used in this experiment determined that, on average, puberty occurred at  $19.6 \pm 3.9$  months and  $22.8 \pm 2.9$  cm of SC (Perez J, unpublished data), values very close to those estimated by the Tanaka model at inflection point. These results provided some information regarding the accuracy of the Tanaka model to estimate the period of highest testicular growth rate in Guzerat bulls and its usefulness in predicting puberty. However, the sigmoidal pattern of SC–age relationship and inflection point estimated by the Tanaka model would be more reliable if there were more observations between birth and 210 days of age (Bilgin et al., 2004).

Regarding MAD, there were small deviations among models, with the lowest values for the Tanaka model indicating better fit average, but at early ages it was the Logistic model that had the lowest MAD. According to Carneiro et al. (Carneiro et al., 2009) the Brody model had the highest MAD, when it was used to describe weight gain of growing Mambrino goats.

From 150 to 1600 days of age the Tanaka and Logistic models were best at describing SC growth in Guzerat bulls. Notably, this period included the ages in which SC had been evaluated in several screening programs, for reproductive traits in males and in genetically related females (Gressler et al., 2000; Silva et al., 2004).

According to Goonewardene et al. (1981) the choice of growth function depends on how it fits the data for each specific age along the growth curve. Thus, similar to the

comparison of SC observed with the predicted, the Tanaka and Logistic models had lower prediction errors and a better fit from weaning to 4 years of age. Therefore, we inferred that the Tanaka model allowed a more appropriate inflection point because it expressed more accurately testis growth at puberty in Guzerat bulls. However, more observations between birth and weaning are needed to improve reliability. Moreover, this model was the best fit to SC data, but, the main disadvantage would be the fact that the biological interpretation is based only on one parameter. Because the Logistic model was the second best model according to fit to actual SC in the range of age evaluated, it was chosen for the environmental effect analysis.

The absolute growth rates based on the first derivative of the Logistic model reached maximum values between 10.6 and 14.5 months. At this stage, the growth rate was remarkably high and positive, reaching the inflection point in the curve. Thereafter, the function changed from increasing to decreasing values. In contrast, in previous studies in Guzerat bulls (Troconiz et al., 1991; Freneau et al., 1997; Barichello et al., 2011), growth rate was lowest from 10.0 and 12.9 months and highest after 13 months of age, with maximum growth rate between 16.0 and 21.9 months.

The AGR and the value of the k parameter estimated by the Logistic model were presumably affected by having bulls from three farms; furthermore, management, year, and season of birth also likely influenced testis growth. In that regard, influences of environmental effects on parameters of the model was confirmed. Furthermore, sexual development and precocity of the bulls

might have been different in the population studied.

There are apparently no reports evaluating the influence of environmental factors on SC growth estimated by a nonlinear model. In the present study, farm, year, and season of birth significantly affected the A and k parameters of the Logistic model. Effect of year, season of birth, and farm on SC have been reported (McManus et al., 2003; Tatman et al., 2004; Pacheco et al., 2007). In this study, bulls born in the dry season had significantly greater value of parameter A (testis size at maturity) than bulls born in the rainy season, in agreement with other reports (Pacheco et al., 2007). The absence of health problems during the first weeks of life and weaning at more favorable times, with greater availability of forage, were probably responsible for better performance of calves born during the dry season (Silva et al., 2001; Pacheco et al., 2007).

Parameter k, which represents maturation rate, is another important feature to be considered, because it indicates growth speed to reach asymptotic adult size. The present study indicated that the increase in SC adult size (A) was accompanied by a decreased SC growth rate (k). Similarly, other authors reported strong genetic antagonism between the A and k parameters, indicating that selection for early maturity would lead to smaller mature size (Brown et al., 1972).

Effects of year and farm on SC were probably consequences of variations in weather and management, which mainly affect the quality and availability of pasture, thereby affecting animal development (Silva et al., 2001; Pacheco et al., 2007; McManus et al., 2003). Several authors have reported

environmental effects on parameters A and k when body growth rate was evaluated in several species (DeNise and Brinks, 1985; Silva et al., 2001; Kratochvílová et al., 2002; Malhado et al., 2009). In Brazil, Nellore cattle have greater body growth rates when born in the dry season, possibly because of the lower incidence of diseases in this season, and because weaning is in the rainy season, the availability of pasture increases (Silva et al., 2004).

Parameters of the nonlinear models can be used to analyze characteristics of growth and determine their biological relevance (Carrijo et al., 1999). Optimization of management techniques in specialized herds is possible with knowledge of nongenetic factors that influence growth rates (Silva et al., 2001); this information is important to help farmers define proper feeding and management strategies (Malhado et al., 2009).

Based on genetic analyses of growth curves, heritability of parameters was low to moderate (Brown et al., 1976; Brown et al.,

1972; DeNise and Brinks, 1985); therefore, mature weight and rate of maturing should respond to selection (Brown et al., 1972). Regardless, genetic and environmental analyses of SC growth curves parameters need to be better investigated.

### **Conclusions**

Scrotal circumference development in Guzerat bulls was characterized by a phase of accelerated growth followed by a decreasing growth rate. More data should be collected at the early and late stages of the animal's life to better describe SC growth in these phases. Regardless, early selection based on testicular size might result in smaller testicular size at maturity. Because growth of SC in Guzerat bulls in this study was significantly influenced by environmental effects, direct comparisons with other breeds are not recommended.

## CHAPTER 2

### SELECTION CRITERIA FOR SEXUAL PRECOCITY IN GUZERAT BULLS RAISED UNDER GRAZING CONDITIONS

#### Abstract

The objectives of the present study were to obtain posterior densities of genetic parameters for scrotal circumference (SC), testicular volume (TV), body weight (BW), and age at puberty, to determine their correlations, and to evaluate the inclusion of these traits as selection criteria for sexual precocity in Guzerat bulls. Two-trait analyses were performed including records of SC, TV, and BW at 365, 450, 550, 650, 730, 850, and 970 d of age with age at puberty of 1,783 Guzerat bulls born between 2000 and 2011. The (co)variance components were estimated using Bayesian methods. Posterior means of heritability ranged from 0.45 to 0.60 for SC, from 0.35 to 0.55 for TV, and from 0.39 to 0.60 for BW. Posterior means of heritabilities for age at puberty using the two-trait analysis with SC ranged from 0.46 to 0.55, those with TV ranged from 0.49 to 0.57, and those with BW ranged from 0.49 to 0.62. The genetic correlation between age at puberty and SC ranged from -0.52 to -0.85, those between age at puberty and TV ranged from -0.33 to -0.66, and those between age at puberty and BW ranged from -0.38 to -0.72. In general, the same trend was observed for the phenotypic correlation between age at puberty and SC, TV, and BW. The selection of the top 10% of young males for SC, TV, or BW caused a decrease in age at puberty, with the most favorable expected correlated response in age at puberty at 650 d of age ( $-119.95 \pm 15.1$  d per generation), 730 d of age ( $-82.20 \pm 20.9$ ), and 850 d of age ( $-93.68 \pm 21.5$ ), respectively. In conclusion, SC, TV, and BW can be used as selection criteria to improve early sexual development in Guzerat bulls, and SC measured at 650 d of age is the most advantageous indicative selection criterion for improvement of age at puberty in Guzerat young bulls.

*Key words:* beef cattle, body weight, genetic parameter, puberty, scrotal circumference, testicular volume

#### Introduction

Zebu breeds (*Bos indicus*) are predominantly used in extensive management systems in most of South America. Unfortunately, Zebu cattle possess several reproductive disadvantages when compared to European breeds (*Bos taurus*), including later sexual development (Fields et al., 1982; Silva-Mena, 1997; Nogueira, 2004; Aponte et al., 2005).

Direct selection for reproduction traits is often difficult to apply, thus making it necessary to identify reproductive traits that are easily measured and correlated to reproductive events. Scrotal circumference (SC) is easily measurable, highly heritable (Yokoo et al., 2007; Boligon et al., 2010; Boligon et al., 2011), positively correlated with seminal quality (Silva et al., 2002; Kealey et al., 2006; Latif et al., 2009) and body weight (BW) (Boligon et al., 2010; Yokoo et al., 2010), and favorably correlated with reproductive performance in females

(Vargas et al., 1998; Martínez-Velázquez et al., 2003; Forni and Albuquerque, 2005; Meirelles et al., 2009).

Some investigators have suggested including measuring testicular volume (TV) in routine andrological assessments of males participating in genetic breeding programs (Unanian et al., 2000) because the two-dimensional measurement of length and width would be a more accurate predictor of TV and weight than the one-dimensional measurement of SC (Bailey et al., 1998). Few studies have estimated the genetic parameters for TV (Boligon et al., 2010; Silva et al., 2011), and the results suggest that a slower genetic gain is therefore expected if this trait is used as a selection criterion (Silva et al., 2011).

Direct selection for male or female sexual precocity traits is not easy. Thus, the objectives of the present study were to obtain posterior densities of genetic parameters for SC, TV, BW measured at different ages and for age at puberty, to determine their correlation, and to evaluate the expected correlated response in age at puberty by the inclusion of SC, TV, and BW in genetic breeding programs for sexual precocity in Guzerat bulls.

## Materials and methods

All procedures performed in this study were approved by the ethics committee of Universidade Federal de Minas Gerais.

### *Animals and Data*

This study was conducted on three farms located in Brasilândia de Minas (17°00'36"S, 46°00'32"W), Carlos Chagas (17°41'30"S, 40°45'15"W), and Unaí

(16°21'43"S, 46°54'09"W) in the state of Minas Gerais, Brazil. The climate classification in these regions is Aw (tropical rainy climate, Köppen classification), with an average temperature of 18°C in the coldest month. The dry season coincides with fall and winter, principally, and these seasons go from April to September. Spring and summer go from October to March. November, December, January, and February receive approximately 60% of the total yearly rainfall. During the nursing period and after weaning (~7 mo old), all Guzerat males were raised under grazing conditions on palisade grass (*Brachiaria brizantha* Stapf cv. Marandu) and bluestem (*Andropogon gayanus* Kunth cv. Planaltina) in the savanna region with water and mineral salt ad libitum. During the dry season the animals were supplemented with salt-type protein or roughage.

Data from 1,783 Guzerat bulls born from 187 sires and 1,125 cows between 2000 and 2011 were used. The following traits were studied: SC, TV, and BW at 365 (SC365, TV365, BW365), 450 (SC450, TV450, BW450), 550 (SC550, TV550, BW550), 650 (SC650, TV650, BW650), 730 (SC730, TV730, BW730), 850 (SC850, TV850, BW850), and 970 d of age (SC970, TV970, BW970). Scrotal circumference was measured in the region of the greatest diameter of the testes and covered two gonads positioned symmetrically side by side, leaving the skin of the scrotum distended. The length and width of each testis were measured with a caliper, and these measurements were used to estimate testicular volume by the formula proposed by Fields et al. (1979):  $TV = 2[(r^2) \times \pi \times L]$ , where  $r = 1/2$  testis width (radius),  $\pi =$  is



equal to 3.141592654, and L = testis length. Testicular measurements and weight were obtained from bulls 305 to 1,030 d of age at intervals of approximately 3 months.

Semen collection by electroejaculation was attempted in the animals when they reached 20 cm SC. Twenty centimeters was the threshold SC below which sperm cell collection was always unsuccessful (Torres-Júnior and Henry, 2005). The interval between semen collections was approximately 3 months, and onset of puberty was considered to be the age when the first mobile spermatozoa were observed in the ejaculate (Garcia et al., 1987; Torres-Júnior and Henry, 2005). When mobile spermatozoa were found 3 months after an unsuccessful attempt, onset of puberty was estimated by interpolation (half the time between the unsuccessful and successful semen collections). It was impossible to measure age at puberty in all animals because some of them had already reached puberty at the first semen collection or they were sold before the end of measurements. Age at puberty in these types of animals was considered missing information. Because of this, we performed two-trait analysis to avoid culling bias (Mrode, 2005).

### Statistical Model

General statistical models for SC, TV, and BW could be represented as:

$$y_{ijkl} = \mu + CG_i + month_j + b(age_k - \overline{age}) + a_1 + e_{ijkl}$$

where  $y_{ijkl}$  represents the y variable (SC, TV or BW) of  $i^{th}$  animal of age  $k$ ;  $i^{th}$

contemporary group and  $j^{th}$  month of birth;  $\mu$  represent a constant;  $CG_i$  represents the fixed effects of  $i^{th}$  contemporary group (farm and year of birth);  $month_j$  represents the  $j^{th}$  fixed effect of month of birth;  $b$  represents the linear regression coefficient of  $age_k$  of the animal at measurements ( $age_k$ );  $\overline{age}$  represents the mean of the age;  $a_1$  represents the random additive genetic effect of animal  $i$ ; and  $e_{ijkl}$  represents the random residual effects. Contemporary groups with less than 2 records were deleted. The statistical model for age at puberty was similar to the previous one with exclusion of the linear regression coefficient.

Under the matrix form, the general model considered in the two-trait analysis was

$$\begin{bmatrix} \tilde{\mathbf{y}}_1 \\ \tilde{\mathbf{y}}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{x}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{x}_2 \end{bmatrix} \times \begin{bmatrix} \tilde{\boldsymbol{\beta}}_1 \\ \tilde{\boldsymbol{\beta}}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{bmatrix} \times \begin{bmatrix} \tilde{\mathbf{a}}_1 \\ \tilde{\mathbf{a}}_2 \end{bmatrix} + \begin{bmatrix} \tilde{\mathbf{e}}_1 \\ \tilde{\mathbf{e}}_2 \end{bmatrix}$$

where  $\tilde{\mathbf{y}}_i$  is a vector with the observations of trait  $i$ ;  $\tilde{\boldsymbol{\beta}}_i$  is a vector with the fixed effect solutions (contemporary groups, month of birth, age);  $\tilde{\mathbf{a}}_i$  is a vector with solutions of the random direct genetic additive effects;  $\tilde{\mathbf{e}}_i$  is a vector with the solutions of random residual effects; and  $\mathbf{x}_1$ , and  $\mathbf{Z}_i$  are

incidence matrices that relate  $\mathbf{y}_i$  with  $\mathbf{\beta}_i$  and  $\mathbf{a}_i$ , respectively.

The assumptions for the random effects were:  $\mathbf{Var}(\mathbf{a}) = \mathbf{G} \otimes \mathbf{A}$  and  $\mathbf{Var}(\mathbf{e}) = \mathbf{R} \otimes \mathbf{I}$ , in which

$$\mathbf{G} = \begin{bmatrix} \sigma_{a1}^2 & \sigma_{a1a2} \\ \sigma_{a1a2} & \sigma_{a2}^2 \end{bmatrix}, \sigma_{a1}^2, \text{ is the additive}$$

genetic variance for trait  $i$  ( $i = 1$  or  $2$ );  $\sigma_{a1a2}$ , is the additive genetic covariance between traits 1 and 2;  $\mathbf{A}$ , is the numerator

relationship matrix;  $\mathbf{R} = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e1e2} \\ \sigma_{e1e2} & \sigma_{e2}^2 \end{bmatrix}$ ,

$\sigma_{e1}^2$ , is the residual variance for trait  $i$ ;  $\sigma_{e1e2}$ , is the residual covariance between traits 1 and 2; and  $\mathbf{I}$ , is the identity matrix of order equal to order of  $\mathbf{y}_i$ . For the

composition of the relationship matrix, a recursive algorithm was used to keep only the individuals with data (1,783), and their ancestors, on pedigree base. In addition, those individuals that did not have data nor were dams of animals with data, that did not have at least one known ancestor, and were linked to only one animal in the database, were also excluded from the pedigree record and the descendant's pedigree. These procedures were repeated until there were no more animals of this type. In this way, a relationship matrix was formed, containing only genealogical data of animals considered informative, i.e., 3,095 animals.

The (co)variance components were estimated by the Bayesian method, using the

INTERGEN program (Cardoso, 2008), in two-trait analysis. Inference was based on Markov Chain Monte Carlo (MCMC) methods with chains of 600,000 cycles with a burn-in period of 100,000 cycles and a thinning interval of 500 cycles. The convergence of the chains was evaluated using the R program with the BOA package (Smith, 2005), which generates convergence diagnostics according to Geweke (1992). In the Geweke test, initial values of the Markov chain are compared with final values of the chain to detect convergence failures. P-values lower than 0.05 indicate the existence of evidence against the convergence of chains.

The correlated response for SC, TV and BW were calculated as:  $\Delta G_{x,y} = i_y \times h_x \times h_y \times r_a \times \sigma_{px}$ , where  $x$ , is the trait indirectly selected;  $y$ , is the trait under selection;  $i_y$ , is the selection intensity considered for trait  $y$  (10% of the males);  $h_x$  is the square-root of the heritability of  $x$ ;  $h_y$  is the square-root of the heritability of  $y$ ;  $r_a$ , is the genetic correlation between  $x$  and  $y$  traits, and  $\sigma_{px}$  is the phenotypic standard deviation of the trait indirectly selected (Falconer and Mackay, 1996).

## Results and Discussion

The number of animals evaluated at each age (SC, TV, or BW), the animals measured at this age with puberty information in some age group, the number and percentage of animals that reached puberty in each age group, and the results of descriptive statistics obtained for testicular and body traits are summarized in Table 1. The means ( $\pm$  SD) of SC, TV, and BW obtained in this work were close to values reported by Trocóniz et al. (1991) and Torres-Júnior and Henry

(2005) for Guzerat bulls. Age at puberty obtained from 821 bulls in this work was  $622.14 \pm 139$  d and was within the range of age at puberty reported in other studies for Guzerat bulls raised under grazing conditions (Garcia et al., 1987; Trocóniz et al., 1991; Torres-Júnior and Henry, 2005).

Because testicular measures and BW obtained in the present study were similar to those obtained in other works, it could be considered that this database is representative for Guzerat bulls raised under grazing condition in South America.

Table 1. Number of observations (n) in each age group, mean and SD for the traits analyzed in Guzerat bulls<sup>1</sup>

Trait	Age, d						
	365 (305 – 425)	450 (426 – 510)	550 (511 – 610)	650 (611 – 710)	730 (711 – 790)	850 (791 – 910)	970 (911 – 1,030)
Mean of age	365.5	469.0	560.7	657.7	746.4	837.9	963.2
SD, d	33.59	24.16	29.36	29.27	22.91	31.30	30.71
Animals measured	851	911	1,035	1,026	809	985	864
Puberty information <sup>2</sup>	432	531	668	676	532	614	520
Pubertal bulls <sup>3</sup>	35 (4.1%)	118 (12.9%)	308 (29.7%)	176 (17.1%)	81 (10%)	71 (7.2%)	32 (3.7%)
Cumulative number <sup>4</sup>	35 (4.3%)	151 (18.7%)	461 (56.2%)	637 (77.6%)	718 (87.5%)	789 (96.1%)	821 (100%)
SC							
n	825	880	987	982	787	966	847
Mean, cm	17.64	19.72	21.64	24.78	27.52	28.75	30.05
SD, cm	2.34	2.75	3.28	3.73	3.98	3.80	3.51
TV							
n	485	664	734	667	532	675	525
Mean, cm <sup>3</sup>	110.64	152.63	216.72	316.11	437.11	502.02	571.73
SD, cm <sup>3</sup>	69.34	76.50	110.31	148.11	180.59	179.55	186.95
BW							
n	688	716	880	830	619	756	708
Mean, kg	189.36	219.87	244.58	281.94	320.80	341.44	376.48
SD, kg	33.59	34.29	38.46	45.82	49.26	56.01	60.04

<sup>1</sup>SC = scrotal circumference; TV = testicular volume; <sup>2</sup>Puberty information = number of animals that were measured in each age group and reached puberty in some age group; <sup>3</sup>Pubertal bulls = number of animals (and percentage) that were measured in that age and reached puberty in that age group; <sup>4</sup>Cumulative number = cumulative number of animals (and percentage) that reached puberty up to each age group during this study.

For every Markov chain the results indicated that the size of chain, burn-in period and thinning interval considered was sufficient to reach convergence. Posterior density statistics of the components of variance for growth and testicular traits obtained by two-trait analysis are shown in Table 2.

Table 2. Posterior density statistics of genetic, environmental and phenotypic variance for scrotal circumference, testicular volume and body weight obtained by two-trait analysis with age at puberty in Guzerat bulls<sup>1</sup>

Trait <sup>2</sup>	Age, d						
	365	450	550	650	730	850	970
<b>SC, cm</b>							
$\sigma_a^2$	1.99	2.97	3.87	6.43	5.16	6.07	5.56
HPD (90%)	[1.12:2.83]	[1.69:4.24]	[1.99:5.58]	[4.36:8.39]	[3.03:7.27]	[3.82:8.08]	[3.33:7.58]
$\sigma_e^2$	2.30	3.53	4.76	4.14	5.73	4.53	4.37
HPD (90%)	[1.66:2.95]	[2.45:4.65]	[3.05:6.04]	[2.61:5.75]	[3.85:7.50]	[2.92:6.29]	[2.71:6.10]
$\sigma_p^2$	4.30	6.51	8.64	10.58	10.89	10.60	9.93
HPD (90%)	[3.89:4.80]	[5.88:7.12]	[7.85:9.51]	[9.59:11.63]	[9.80:12.01]	[9.45:11.54]	[8.87:10.92]
<b>TV, cm<sup>3</sup></b>							
$\sigma_a^2$	1,006.42	2,253.11	4,227.63	8,385.52	10,789.22	13,432.87	8,181.92
HPD (90%)	[431.98:1,669.43]	[1,287.41:3,349.93]	[2,241.06:6,083.02]	[4,688.25:12,313.46]	[4,996.45:15,990.45]	[7,930.69:20,333]	[3,641.90:13,938.77]
$\sigma_e^2$	1,841.29	3,109.62	5,991.28	9,499.96	12,878.35	10,675.80	15,677.62
HPD (90%)	[3,958.20:8,757.92]	[3,747.25:8,859.21]	[3,578.10:8,442.32]	[4,307.70:9,652.62]	[4,470.56:9,473.43]	[3,608.60:8,586.60]	[4,548.64:9,751.23]
$\sigma_p^2$	2,847.70	5,362.73	10,218.91	17,885.48	23,667.56	24,108.67	23,859.54
HPD (90%)	[2,499.53:3,215.97]	[4,743.68:5,963.68]	[9,222.88:11,388.33]	[16,062.90:19,917.70]	[20,861.16:26,795.23]	[21,154.62:26,940.34]	[20,981.23:26,777.93]
<b>BW, kg</b>							
$\sigma_a^2$	365.22	390.17	598.53	783.01	997.32	1,301.06	1,511.38
HPD (90%)	[211.53:539.73]	[219.56:569.51]	[333.17:867.54]	[447.89:1,143.28]	[562.27:1,413.57]	[841.84:1,744.34]	[964.80:2,037.84]
$\sigma_e^2$	562.21	584.63	712.96	943.95	880.20	878.73	978.85
HPD (90%)	[401.20:693.37]	[432.41:724.80]	[506.77:933.29]	[639.72:1,214.07]	[555.44:1,222.79]	[586.91:1,269.27]	[597.25:1,449.33]
$\sigma_p^2$	927.43	974.80	1,311.50	1,726.97	1,877.54	2,179.80	2,490.24
HPD (90%)	[824.94:1,024.76]	[879.39:1,102.37]	[1,179.79:1,446.24]	[1,555.94:1,901.96]	[1,680.78:2,142.58]	[1,960.46:2,457.36]	[2,213.82:2,765.28]

<sup>1</sup> $\sigma_a^2$  = mean additive genetic variance;  $\sigma_e^2$  = mean environmental variance;  $\sigma_p^2$  = mean phenotypic variance; HPD = highest posterior density interval (90%). <sup>2</sup>SC = scrotal circumference; TV = testicular volume.

The posterior means (PM) of heritability for SC at different ages were considered to have a moderate to high magnitude (Figure 1A) and were larger than estimates reported in the literature for Zebu cattle (Boligon et al., 2007; Yokoo et al., 2007; Frizzas et al., 2009; Boligon et al., 2010, 2011). The PM of heritability for SC650 was greater than those obtained for the other ages. Frizzas et al. (2009) and Boligon et al. (2010) estimated greater heritability for SC measured at 550 d when compared to those obtained at weaning or at 365 d of age in Nellore cattle. In our study, heritability at weaning was not estimated because there were few records for this age.

In contrast, Yokoo et al. (2007) reported greater heritabilities at 450 (0.53) than at 550 d of age. Similarly, in the present study, PM of heritability for SC365 and SC450 were greater than those obtained for SC550. Because of the magnitude of heritability, Silva et al. (2011) suggested that direct selection for SC at 550 d of age in Nellore cattle could result in rapid genetic progress for this trait; however, PM of heritability obtained for SC at 550 d was lower than that obtained for the same age in the present study.

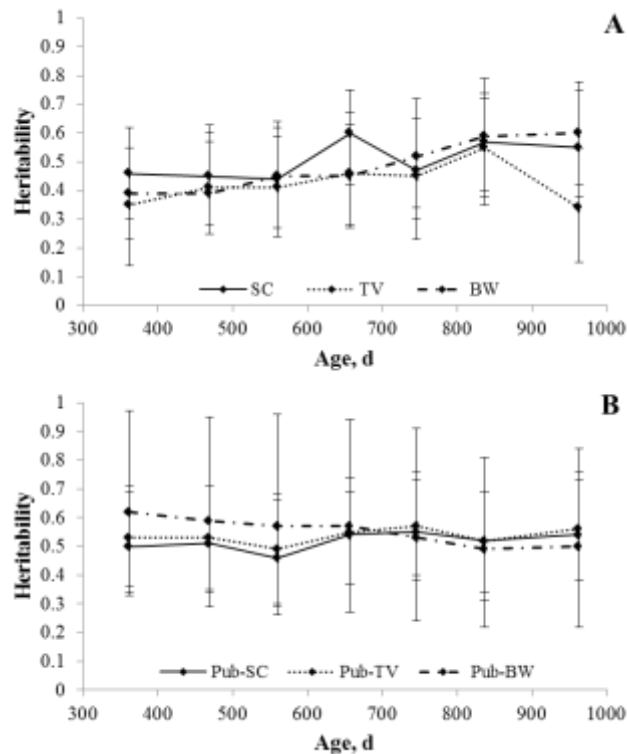


Figure 1. Posterior means and highest posterior density intervals (90%) obtained for heritability of scrotal circumference (SC), testicular volume (TV) and BW at different ages in two-trait analysis with age at puberty (A). Posterior means and highest posterior density intervals (90%) obtained for heritability of age at puberty (Pub) in two-trait analysis with SC, TV and BW in Guzerat bulls (B).

Yokoo et al. (2007) indicated the possibility to select animals with greater testicular size using only SC measured at 450 d of age because this trait will provide greater response to selection. Heritability estimates by Yokoo et al. (2007) for SC450 were within the 90% highest posterior density (HPD) interval obtained for this trait. In the present study, the heritability estimate at 650 d was markedly greater than that obtained at other ages; hence, this finding and the phenotypic variances obtained suggest that considering the same intensity of selection at all ages, the response to selection for SC in Guzerat bulls should be greater at 21 months. Coulter et al. (1987) suggested that precocious beef breeds have high SC heritability at young ages and that the age when optimum response to selection for testicular size occurs will vary with breed. This could explain the differences in SC heritability reported in the literature for Nellore bulls (Yokoo et al., 2007; Boligon et al., 2010; Silva et al., 2011) and those obtained in this study for Guzerat bulls.

Posterior means of heritability estimates for TV were of moderate to high magnitude and increased from 365 to 850 d of age (Figure 1A). The PM of heritability for TV obtained in the present study was greater than that reported by Toelle and Robinson (1985), Lunstra et al. (1988), Quirino et al. (1999), Boligon et al. (2010), and Silva et al. (2011). Pachymeter is (Digimess, São Paulo, SP, Brazil) the most widely used tool to measure the width and length of both testes, and these measurements are necessary to obtain TV. However, the inaccuracies of the measurements and the complicated equations could be associated to errors in the measurements of this trait.

Posterior means of heritability for BW remained constant between 365 and 450 d of age (0.39) and between 550 and 650 d of age

(0.45) and increased from 730 to 970 d of age (Figure 1A). Heritability estimates for BW in this study were larger than those reported by Forni and Albuquerque (2005) and Boligon et al. (2010) for Nellore bulls but were less than those reported by Yokoo et al. (2007) for BW365, BW450, and BW550.

Posterior density statistics of the components of variance for age at puberty obtained by two-trait analysis are shown in Table 3. Wolf et al. (1965) defined puberty as the age when a bull first produces an ejaculate containing  $50 \times 10^6$  sperm of which  $\geq 10\%$  are progressive, and Garcia et al. (1987) used the first mobile spermatozoa observed in the ejaculate as the onset of puberty. Thus, frequent attempts of seminal collection are necessary to identify the onset of puberty in bulls. Posterior means of heritability estimates for age at puberty in this study were of moderate to high magnitude (0.46 to 0.62) and are shown in Figure 1B. The heritability obtained by two-trait analysis for age at puberty with testicular traits (SC and TV) increased at 365 and 450 d of age and decreased at 550 d. At 650 d of age the heritability of age at puberty increased in the two analyses. The greater heritability obtained for age at puberty in two-trait analyses with BW was observed at 365 d, followed by a decline to 970 d of age. However, the HPD (90%) were overlapping in all the analyses. References for heritability for age at puberty in bulls were not found, and possibly, this is the first work that reports this information. The high heritability of age at puberty in bulls found in the present study indicated that this trait will respond to selection. However, puberty is a trait difficult to measure in large herds because of the need for frequent semen collections; therefore, more easily measurable traits seem to be a more feasible alternative to use as selection criteria for early sexual development.

The PM of genetic and phenotypic correlations between the traits studied with age at puberty and the HPD (90%) interval are shown in Figure 2. The genetic correlations observed between SC and age at puberty at all ages were negative and strong and were greater at 650 d of age compared to those obtained at other ages (Figure 2A). After 650 d of age, the genetic correlation between SC and age at puberty decreased, possibly because Guzerat bulls in this study reached puberty at 622.14 d of age. The phenotypic correlations were also negative and greater between 450 and 650 d than at other ages (Figure 2B). The negative and high genetic correlation observed between SC and age at puberty in this study suggests that the expression of these traits is mainly influenced by the same genes and that the selection for greater SC could produce a decrease in age at puberty.

Posterior means of expected correlated responses to selection are shown in Table 4. The selection for 10% of the young males with high SC is expected to provide a decrease in age at puberty, and the most favorable expected correlated response between these two traits was at 650 d of age. Boligon et al. (2010) and Silva et al. (2011) suggested that the selection of males based on SC can be made between 12 and 18 months of age, and SC at 18 mo used as a selection criterion may promote favorable correlated responses in TV, semen quality, and satisfactory breeding soundness evaluation in Nellore bulls (Silva et al., 2011). However, in these works the genetic parameters for SC at 650 d were not estimated.

In Guzerat bulls, faster testicular growth rate ranged between 13 and 16 months of age (Garcia et al., 1987; Torres-Júnior and Henry, 2005; Loaiza-Echeverri et al., 2013), and in this phase, the genes related to sexual development

might be active. The high PM of genetic correlation between SC and age at puberty at 650 d of age found in this study could be because at this age the genes related to the onset of puberty were expressed and at this stage of development it is possible to identify early or late maturing bulls.

Lunstra and Echternkamp (1982) found a simple correlation between SC, sperm concentration (0.64), and progressive motility (0.74), indicating that SC may be useful for describing pubertal status in groups of young bulls, regardless of breed. Brito et al. (2004), working with Nellore and Canchim bulls, suggested the use of yearling SC to select bulls for sexual precocity.

Posterior means of genetic correlation between TV and age at puberty were negative and strong and greater for TV750 compared to those obtained at yearling and other ages (Figure 2A). The phenotypic correlation was greater at 450 d and decreased to 970 d of age. According to Silva et al. (2011) and as shown in the present study, TV could be used in routine andrological assessments of males participating in genetic breeding programs. However, great care must be taken when measuring width and length of both testes because some structures surrounding the testicle can interfere with the accuracy of the measurements.

Posterior means of expected correlated response in age at puberty from selection for TV are shown in Table 4. The selection of 10% of the males for high TV is predicted to provide a decrease of age at puberty, and the most favorable expected correlated response was at 730 d age. In contrast, Boligon et al. (2010) suggested that the best age to measure this trait as a selection criterion is between 365 and 550 d of age, but in that work, correlations of

testicular traits with age at puberty were not estimated. In the present work, the PM of genetic correlations between TV and age at puberty were lower than those obtained for SC and age at puberty for all ages. In addition, the expected correlated responses for age at puberty were greatest with SC.

Posterior means of genetic correlation between BW and age at puberty ranged from moderate to high and remained constant until 450 d of age. From 550 to 850 d of age, the genetic correlation increased (Figure 2A). The phenotypic correlations between BW and age at puberty were of moderate magnitude (-0.30 to -0.38). The greatest favorable estimated correlated response for age at puberty by BW selection was at 850 d of age.

These results indicate that more favorable correlated responses for age at puberty can be obtained when testicular traits are used as selection criteria. Also, SC is the trait that

produced the most favorable expected correlated response at the youngest age.

### **Implications**

The reproductive and growth traits evaluated in this study show genetic and phenotypic variability that supports the inclusion of these traits in selection programs for South American Guzarat cattle. The genetic correlations between SC, TV, and BW with age at puberty in males suggest that these traits can be used as selection criteria for sexual precocity. However, in view of the difficulty in measuring TV and the favorable association between SC and age at puberty, SC seems to be the most suitable trait to use as a selection criterion for sexual precocity, and therefore, measuring TV seems not to be necessary. The use of scrotal circumference at 650 d of age as a selection criterion should promote a favorable correlated response for age at puberty.



Table 3. Posterior density statistics of genetic, environmental and phenotypic variance for age at puberty obtained by two-trait analysis with scrotal circumference, testicular volume and body weight in Guzerat bulls<sup>1</sup>

Trait <sup>2</sup>	Age, d						
	365	450	550	650	730	850	970
Pub-SC, d							
$\sigma_a^2$	6,005.09	6,164.03	5,648.17	6,822.90	6,802.90	6,331.65	6,507.03
HPD (90%)	[3,844.13:8,677.30]	[3,592.82:8,572.86]	[3,114.28:8,226.25]	[4,332.51:9,141.20]	[4,163.20:9,252.26]	[3,963.18:8,739.36]	[4,244.23:9,117.86]
$\sigma_e^2$	5,793.08	5,793.09	6,333.70	5,692.80	5,376.08	5,663.57	5,478.68
HPD (90%)	[3,665.85:7,640.00]	[3,530.74:7,726.30]	[4,091.82:8,449.69]	[3,863.97:7,761.10]	[3,345.06:7,474.39]	[3,622.37:7,615.77]	[3,330.14:7,259.18]
$\sigma_p^2$	11,798.17	11,957.12	11,981.87	12,514.88	12,178.98	11,995.22	11,985.71
HPD (90%)	[10,522.87:12,921.20]	[10,808.31:13,237.52]	[10,842.82:13,410.08]	[11,049.69:13,821.77]	[10,997.48:13,526.36]	[10,839.16:13,353.57]	[10,772.78:13,240.71]
Pub-TV, d							
$\sigma_a^2$	6,333.78	6,405.34	5,803.86	6,682.20	6,833.24	6,158.00	6,778.55
HPD (90%)	[3,958.20:8,757.92]	[3,747.25:8,859.21]	[3,528.10:8,442.32]	[4,307.70:9,652.62]	[4,470.56:9,473.43]	[3,608.60:8,586.94]	[4,548.64:9,751.23]
$\sigma_e^2$	5,470.84	5,616.52	5,882.76	5,326.52	5,116.63	5,623.71	5,088.82
HPD (90%)	[3,374.36:7,480.40]	[3,464.19:7,578.17]	[3,641.84:7,899.59]	[3,143.49:7,364.91]	[3,079.04:7,042.57]	[3,623.18:7,809.46]	[3,065.82:7,205.91]
$\sigma_p^2$	11,804.62	12,021.86	11,686.62	12,008.72	11,949.87	11,781.71	11,867.37
HPD (90%)	[10,624.50:13,045.70]	[10,796.28:13,218.58]	[10,611.80:12,972.17]	[10,853.25:13,409.69]	[10,717.02:13,287.77]	[10,558.14:13,033.50]	[10,757.16:13,110.98]
Pub-BW, d							
$\sigma_a^2$	7,470.47	7,067.32	6,893.45	6,822.77	6,337.23	5,754.37	6,012.60
HPD (90%)	[3,196.83:12,328.41]	[3,088.66:12,205.60]	[2,576.93:12,399.58]	[2,533.78:11,727.10]	[2,325.03:11,743.20]	[2,426.30:9,954.23]	[2,325.48:10,431.43]
$\sigma_e^2$	4,373.88	4,730.16	4,990.33	4,971.45	5,388.74	5,809.95	5,657.66
HPD (90%)	[426.81:7,573.22]	[751.26:8,063.24]	[687.87:8,545.90]	[931.03:8,337.95]	[1,043.69:8,612.04]	[2,261.08:8,828.79]	[1,908.80:8,772.84]
$\sigma_p^2$	11,844.35	11,797.47	11,883.78	11,794.22	11,725.97	11,564.33	11,670.26
HPD (90%)	[10,453.85:13,594.33]	[10,313.51:13,217.58]	[10,412.65:13,552.04]	[10,440.44:13,328.53]	[10,432.46:13,333.42]	[10,244.44:12,934.67]	[10,229.05:12,921.32]

<sup>1</sup> $\sigma_a^2$  = mean additive genetic variance;  $\sigma_e^2$  = mean environmental variance;  $\sigma_p^2$  = mean phenotypic variance; HPD = highest posterior density interval (90%).<sup>2</sup>

Pub = age at puberty; SC = scrotal circumference; TV = testicular volume.

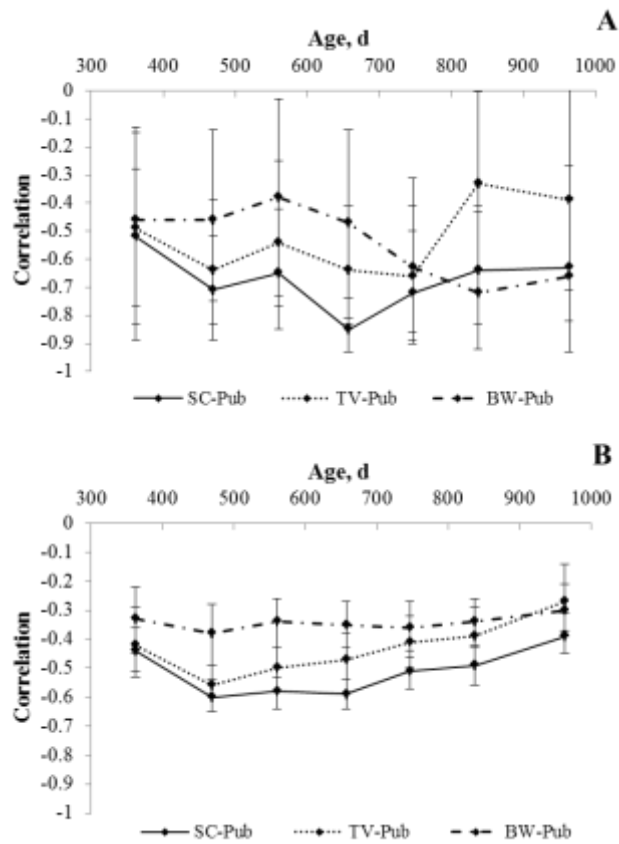


Figure 2. Posterior means and highest posterior density intervals (90%) for genetic (A) and phenotypic (B) correlation between scrotal circumference (SC), testicular volume (TV) and BW with age at puberty (Pub) in Guzerat bulls.

Table 4. Posterior density statistics of correlated genetic responses per generation for age at puberty by the scrotal circumference, testicular volume and body weight selection in Guzerat bulls<sup>1</sup>

Trait <sup>2</sup>	Age, d						
	365	450	550	650	730	850	970
SC, d	-63.08	-85.35	-73.39	-119.95	-90.93	-86.04	-84.95
HPD (90%)	[-105.18:-24.26]	[-127.22:-49.42]	[-115.49:-35.76]	[-154.37:-84.47]	[-128.05:-51.17]	[-124.50:-50.85]	[-122.49:-46.14]
TV, d	-52.16	-74.73	-60.83	-80.06	-82.20	-44.07	-42.22
HPD (90%)	[-97.51:-8.18]	[-118.97:-36.26]	[-100.02:-18.73]	[-116.79:-37.77]	[-121.00:-39.77]	[-94.32:-0.49]	[-87.71:-0.434]
BW, d	-56.36	-55.72	-48.55	-58.86	-80.59	-93.68	-86.92
HPD (90%)	[-102.24:-8.38]	[-104.36:-1.09]	[-97.03:-0.10]	[-108.28:-13.79]	[-129.64:-34.72]	[-135.26:-53.59]	[-132.41:-34.04]

<sup>1</sup>HPD = highest posterior density interval (90%).

<sup>2</sup>SC = scrotal circumference; TV = testicular volume.

## CHAPTER 3

### PROBABILITY OF REACHING PUBERTY IN FUNCTION OF THE SCROTAL CIRCUMFERENCE AND AGE IN GUZERAT BULLS RAISED UNDER GRAZING CONDITIONS

#### Abstract

The aim of the present work was study the association of the scrotal circumference (SC) and age with the probability of reaching puberty in Guzerat bulls raised under grazing conditions. A total of 10290 SC measures from 1661 Guzerat bulls were used. The logistic regression model was utilized for the study of association between the response variable pubertal stage with the independent variables age and SC. Scrotal circumference increase resulted in the increase of the odds puberty occurrence in Guzerat bulls. The Odds ratio indicated that the increase in one unit of SC can increase in 52% the odds of the Guzerat bulls be pubertal, and the increase in one unit of age can increase in 0.3% the odds of bulls be pubertal. Until 20 cm of SC, only 15% of Guzerat bulls reached puberty, at 22 cm this percentage increased to 30%, at 24 cm of SC the probability was 50% and at 28 cm the probability of Guzerat bulls reaching puberty was 84%. The probability of bulls reaching puberty by age was 55% at 450 days, at 550 days of age increase to 64%, reached 70% after 100 days approximately. Until 657 days of age the probability was 72%, and until 1020 days of age was 90%. We concluded that probability of Guzerat bulls reaching puberty will be better estimated having a reference of selection animal with scrotal circumference between 22 and 24 cm.

#### Introduction

Among the actions that lead a system of cattle production to be more productive, includes selecting of animals for sexual precocity, which can increase the length of the productive life of animals in the herd and reduce the interval between generations (Meirelles et al., 2009). Scrotal circumference has favorable genetic correlation with age at puberty in bulls (Loaiza-Echeverri et al., 2013), is an easy trait to be measure and presented moderate to high heritability (Faria et al., 2008; Boligon et al., 2010; Silva et al., 2011; Loaiza-Echeverri et al., 2013). Furthermore, scrotal circumference is favorably correlated with reproductive female traits as age at puberty, age at first calving and interval between calving (Vargas et al., 1998; Martínez-Velázquez et al., 2003; Meirelles et

al., 2009). It has been demonstrated that bulls with higher scrotal circumference at early ages reach puberty faster and has better sperm quality that those bulls of normal or late sexual development (Moser et al., 1996; Brito et al., 2004; Siddiqui et al., 2008). Because that, scrotal circumference have been used as selection criterion for sexual precocity in beef bulls.

European beef cattle breeds reach puberty around 300 days of age (Lunstra e Echterkamp, 1982; Jiménez-Severiano, 2002); while in zebu breeds, puberty occurs approximately at 480 days of age (Aponte et al., 2005; Torres e Henry, 2005; Freneau et al., 2006). Nevertheless, variations in age at puberty exist between and within breeds, and these variations are higher in herds without

selection for this trait (Lunstra and Echterkamp, 1982; Freneau et al., 2006). Because of that the age range for sexual precocity selection suitable for a determinate breed could be non-suitable for another breed.

In Guzerat bulls it was observed that some animals with 20 cm of scrotal circumference presented spermatozoa with motility at semen collection by electroejaculation while in other bulls, sperm cells were found only with larger scrotal circumference, indicating also that exist variability in scrotal circumference at puberty in animals from this breed (Torres e Henry, 2005; Perez et al., 2012). However, it has been reported for European cattle breeds that there is no significant differences in scrotal circumference at puberty, even when differences in the age and weight at puberty were found (Lunstra e Echterkamp, 1982; Lunstra e Cundiff, 2003).

Due to that, it is important for each breed and breeding system to determinate the better age range and scrotal circumference size at which selection for sexual precocity is most suitable. Prediction of puberty could be an auxiliary tool for the identification of sexual precocity in bulls, due to that, this permit selection of sires at more early ages and reduces cost of breeding animal with poor reproductive performance. Thus, the objective of this work was study the association of the scrotal circumference and age with the probability of reaching puberty in Guzerat bulls raised under grazing conditions.

### Material and methods

A total of 10290 scrotal circumference records from 1661 Guzerat bulls, aged between 192 and 1525 days (Figure 1), born from 188 sires and 1075 cows between 2000 and 2011, were used. This study was conducted on three farms located in Brasilândia de Minas (17°00'36"S,

46°00'32"W), Carlos Chagas (17°41'30"S, 40°45'15"W), and Unai (16°21'43"S, 46°54'09"W) in the state of Minas Gerais, Brazil. The climate classification in these regions is Aw (tropical rainy climate, Köppen classification), with an average temperature of 18°C in the coldest month. The dry season coincides with fall and winter, principally, and these seasons go from April to September. Spring and summer go from October to March. November, December, January, and February receive approximately 60% of the total yearly rainfall. During the nursing period and after weaning (~7 mo old), all Guzerat males were raised under grazing conditions on palisade grass (*Brachiaria brizantha* Stapf cv. Marandu) and bluestem (*Andropogon gayanus* Kunth cv. Planaltina) in the savanna region with water and mineral salt ad libitum. During the dry season the animals were supplemented with salt-type protein or roughage.

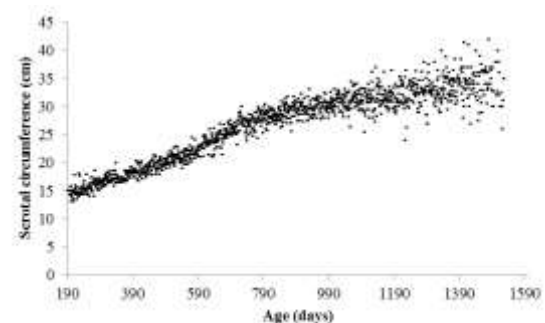


Figure 1. Mean of the scrotal circumference measure in each age in Guzerat bulls

Semen collection by electro-ejaculation was attempted in the animals when they reached 20 cm of scrotal circumference. Twenty centimeters was the threshold scrotal circumference below which sperm cell collection was always unsuccessful (Torres-Júnior and Henry, 2005). The interval between semen collections was approximately 3 months, and onset of puberty was considered to be the

age when the first mobile spermatozoa were observed in the ejaculate (Garcia et al., 1987; Torres-Júnior and Henry, 2005). When mobile spermatozoa were found 3 months after an unsuccessful attempt, onset of puberty was estimated by interpolation (half the time between the unsuccessful and successful semen collection).

Animals that presented spermatozoa with motility greater than 10% and sperm concentration greater than 2 (measure subjectively in scale from 1 to 5, when 5 is the highest concentration) in the first semen collection were considered pubertal animals, had their data included in the analysis of probability, but were not used to calculate the mean of age at puberty, because of the high sperm concentration do not permit calculate the age at the first sperm. Of 1661 evaluated bulls, 815 reached puberty during the collection data.

The logistic regression model calculated by the procedure LOGISTIC of SAS system (SAS Institute, 2001; SAS Institute, Cary, NC, USA) was utilized for the study of association between the response variable pubertal stage with the independent variables: age and scrotal circumference. Pubertal stage is a variable with binomial character, when the animal was considered pubertal (success = 1) or non-pubertal (unsuccessful = 0) at evaluation moment; for this, all animals received a classification each three months. Classification 1 was obtained only when motile spermatozoa were found in the ejaculate. Thus, 6035 records obtained classification 1 and 4255 classification 0.

For the calculation of the probability of the animal reaching puberty the following statistic model was used:

$$\ln\left(\frac{P_{ijklm}}{1-P_{ijklm}}\right) = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2j} + \beta_3 x_{3k} + \beta_4 x_{4l} + \varepsilon_{ijklm}$$

Where  $P_{ijklm}$  is the probability of the animal  $m$  be pubertal, with age  $i$ , scrotal circumference  $j$ , contemporary group (year and farm of birth)  $k$  and born in the month  $l$ ;  $\beta_0$  is a general constant present in all records;  $\beta_h$  ( $h=1, 2, 3$  e  $4$ ) are the regression coefficient associated to the regression variables  $X_h$ ; and  $\varepsilon_{ijklm}$  is the random error associated to each record.

Estimative of parameters ( $\hat{\beta}_h$ ) were obtained for the method of maximum likelihood and the final model was choose by the Stepwise option of the LOGISTIC procedure of SAS system (SAS Institute, 2001; SAS Institute, Cary, NC, USA). Stepwise procedure was used for select the most appropriate model for pubertal stage prediction, this procedure identify the variable that provides the best fit to the records (high  $R^2$ ). After this, the second variable that provides better fit was included into the model. Significance of the regression coefficients was considered to evaluate the need to exclude any variable. This step was repeated until there were no more variables to be added or excluded of the model (Freund & Littell, 2000).

After estimate of  $\beta_0, \dots, \beta_4$ , probability of reaching puberty ( $P(X=1)$ ) was estimated by the equation:

$$\hat{P}(X=1) = \frac{1}{1 + e^{-(\hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_2 + \hat{\beta}_3 x_3 + \hat{\beta}_4 x_4)}}$$

where:  $x_1, \dots, x_4$  were the reference values for the independent variables included into the final model. In the present situation, to facilitate the

visualization of the variable effects of interest over the probability of reaching puberty were considered the reference values of contemporary group and month of birth with the larger number of records, and the interval of scrotal circumference and age evaluated in the data base. For the study of scrotal circumference effect on probability of reaching puberty separately, it was used 550 days as reference value for age; in the same way, for the study of age effect on probability of reaching puberty separately, it was used 25.37 cm as reference value for scrotal circumference.

The odds ratio  $\left( \frac{p(\text{success})}{p(\text{unsuccessful})} \right)$  associate to each regressor variable was estimated by  $e^{\hat{\beta}_h}$  that define the changes in the odds ratio when there is an alteration of one unit in the regressor variable (Freund and Littell, 2000).

Statistics used for validation of the models was the percentage of observed and predicted concordant pairs. This procedure consists in the comparison of the probabilities between the observed and predicted responses considering all possibilities of observation pairs ( $y_i, y_j$  where  $i \neq j$ ), such that the observed responses for the  $y_i$ 's equalled 1 (success) and the observed responses for the  $y_j$ 's equalled 0 (unsuccessful). There were  $n_i \times n_j$  such pairs of observations (number of nonpubertal  $\times$  number of pubertal bulls) that were classified as concordant when  $\hat{P}_i > \hat{P}_j$ , and discordant if  $\hat{P}_i = \hat{P}_j$ . The proportion for each model was calculated in relation to the total number of pairs  $n_i \times n_j$  (Bergmann & Hohenboken, 1992).

Significance level for effects of the variation sources was calculated by the test of maximum likelihood, that test consist in comparison

between the value obtained of the likelihood function in the full and the reduced model.

## Results and Discussion

Mean of age at puberty and scrotal circumference at puberty for Guzerat bulls evaluated in this study were  $623 \pm 140.64$  days and  $24.8 \pm 2.7$  cm, respectively. Torres and Henry (2005) and Osorio et al. (2012) working with part of the records used in the present study, reported values of  $564 \pm 63$  and  $588 \pm 117$  days for age at puberty, and  $24.2 \pm 2.4$  cm and  $22.8 \pm 2.9$  cm for scrotal circumference at puberty, respectively. Troconiz et al. (1991) reported similar results for Guzerat bulls raised under grazing conditions in Venezuela,  $540 \pm 60$  days and  $25.6 \pm 2.2$  cm for age and scrotal circumference at puberty. The high value of standard deviation of age at puberty found in this study, when compared with the other authors, can be due to the fact of the records are from several farms, and possibly genetic and environmental factors are influencing the sexual development of bulls. Nevertheless, despite the differences in age at puberty reported in the present study and the values reported by Troconiz et al. (1991), Torres and Henry (2005) and Osorio et al. (2012), scrotal circumference at puberty was similar between the works, and this could be suggesting that this trait is a good indicator of age at puberty in Guzerat bulls, and this was reported by other authors in European and Zebu breeds (Lunstra et al., 1978; Brito et al., 2004).

Any of the independent variables considered in this study were eliminated, according to the likelihood test at significance level of 5%. Based in the significance level, the inclusion order of the variables was: scrotal circumference, contemporary group, age and month of birth.

Contemporary group and month of birth presented significant effect ( $P < 0.05$ ) on the probability of reaching puberty, due to this they were maintained into the statistic model for ensure the better fit of the model to the records. Significant effect on probability of reaching puberty of these two environmental factors was expected due to the fact of the records are from different farms and regions, and differences in management and food availability exist between the farms and within the same farms in different seasons. However, the main objective of this work was not to evaluate the effect of

these variables on probability of reaching puberty, because of that, those results will not be discussed.

The regression coefficient of the two variables (age and scrotal circumference) evaluated in conjunct were positive (Table 1), indicating that the increase of one unit in each trait would increase the chance of animals reaching puberty.

Table 1. Probability of reaching puberty in Guzerat bulls using scrotal circumference and age as regression variables in the model.

Regressor variables	Regression coefficiente		Odds ratio	Confidence intervals		% of pairs <sup>1</sup>	
	$\hat{\beta}_0$	$\hat{\beta}$		Conc.	Disc.		
SC		0.4203	1.522	1.483	1.563	95.5	4.4
Age	-12.8174	0.00349	1.003	1.003	1.004		

SC = scrotal circumference;  $\hat{\beta}_0$  = intercept;  $\hat{\beta}$  = regressor variables; 1. Predicted probability-observed response concordant and discordant.

Scrotal circumference increase and age increase resulted in the increase of the odds of puberty occurrence in Guzerat bulls. The Odds ratio of reaching puberty by scrotal circumference size was greater when compared to the odds ratio of reaching puberty by the age, and this indicates that the largest scrotal circumference increased the odds of reaching puberty more than the age. In this way, a bull with large scrotal circumference has 52% more odds of reaching puberty faster than bulls with smaller scrotal circumference.

The odds of the animal be pubertal was higher when scrotal circumference increased; however, comparisons between regressor variables cannot be made because the units of measure are different. Nevertheless, despite the moderate to high heritability of scrotal circumference and the expected favorable response to selection

(Boligon et al., 2011; Silva et al., 2011; Loaiza-Echeverri et al., 2013), increase of one centimeter in scrotal circumference is a slow process. Costa et al. (2004) found in a Nellore herd, in which selection for larger scrotal circumference was applied, that the genetic trends for this trait was 0.101 cm/year, while Cyrillo et al. (2001) reported genetic trend of 0.31 cm/year in bulls of the same breed.

Siddiqui et al. (2008) evaluating records from crossed bulls found significant effect of the scrotal circumference ( $P < 0.05$ ), weight and body condition on the moment in that each bull reached puberty, and this do not happened with the age. According to the odds ratio reported by these authors, increase in one unit of scrotal circumference increase in 16% the odds of the animal be pubertal.



Brito et al. (2004) reported that Nellore bulls reached puberty with scrotal circumference of  $22.5 \pm 0.6$  cm and  $526.7 \pm 12.3$  days of age, while late sexual development bulls reached puberty with  $26.2 \pm 0.6$  cm and  $673.3 \pm 19.1$  days; these values were similar to the scrotal circumference and age at puberty founded in the present work for Guzerat bulls. In the same way, Freneau et al. (2006) reported that Nellore bulls raised in grazing conditions reached puberty approximately at 450 days of age, with 25.2 cm of scrotal circumference. The larger age and lower scrotal circumference of Guzerat bulls at puberty could be indicating that sexual development in Guzerat bulls is slower when compared to sexual development in Nellore bulls. Nevertheless, in the present study it was found variability in the age at puberty. Some

bulls reached puberty around 300 days of age and 21 cm of scrotal circumference, approximately, while some bulls reached puberty with age higher than 1000 days, and scrotal circumference between 24 and 32 cm.

According to Lima et al. (2011), Nellore bulls selected at 12 months of age having scrotal circumference 5% larger than the mean, reached puberty from 2 to 3 months before the rest of contemporary group, and presented higher scrotal circumference and weight gain from 12 to 18 months, when compared with the unselected bulls. Thereby, these authors suggested that scrotal circumference measure at 12 months of age is a suitable marker to predict age at puberty of bulls, and consequently, this measure could be used as selection criterion for early sexual development.

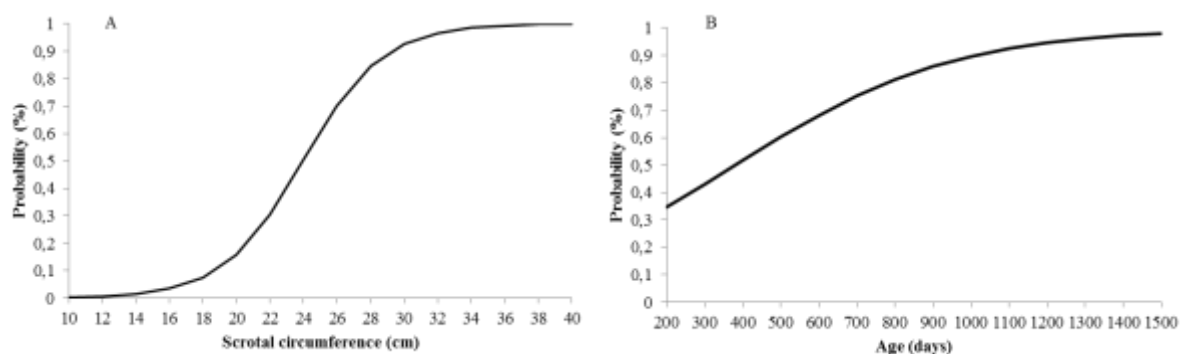


Figure 2. Probability of reaching puberty in Guzerat bulls in function of scrotal circumference (A) and age (B).

Probability of reaching puberty increased with the increase of scrotal circumference (Figure 2, A). According to the results obtained in the present work, until 20 cm of scrotal circumference, only 15% of Guzerat bulls reached puberty; nevertheless, at 22 cm this percentage increased to 30, and at 24 cm of scrotal circumference probability of bulls reaching puberty was 50%.

In the same way, Brito et al. (2004) suggested that 19 cm of scrotal circumference in Nellore bulls and 24 cm in Canchin bulls at 365 days of age can be used as criterion selection for early sexual development; on the other hand, our results showed that Guzerat bulls that reached faster 24 cm of scrotal circumference could be animals of early sexual development. In the same way, even Guzerat males not being animals of fast scrotal circumference growth,

the present results indicated that at 24 cm of scrotal circumference, half of them are pubertal.

At 28 cm of scrotal circumference, probability of Guzerat bulls reaching puberty is 84%. These results are close with the results reported by Perez et al. (2012), where 91.1% of Guzerat bulls with 28 cm of scrotal circumference, and age between 24 and 28 months had reached puberty.

Torres and Henry (2005) evaluating part of the population used in the present study, founded that within a group of 64 Guzerat bulls with scrotal circumference between 23 and 25.5 cm, 79% of them reached puberty. In our study, mean of scrotal circumference at puberty was  $24.8 \pm 2.7$  cm, indicating that in the whole group the scrotal circumference at puberty was maintained.

Considering now the probability of bulls reaching puberty by the age, we found that until 450 days of age, probability of reaching puberty was 55%, at 550 days of age increase to 64% reached 70% after 100 days approximately (Figure 2, B). Until 657 days of age 72% of bulls reached puberty, and until 1020 days of age the probability was 90%.

Comparing the two figures of probability, it can be inferred that with low increases in scrotal circumference is possible obtain a significant increase in the probability of reaching puberty; whiles with age, in an interval of 100 days the increase in the probability of reaching puberty will be less than one unit. Nevertheless, age is a variable that increase independent of the applied selection; whiles significant changes in scrotal circumference in a generation without genetic selection for this trait could be late, additionally, environmental factors, as nutrition, could be influenced this changes.

Probability of reaching puberty increase with the increase of scrotal circumference; however, when inflection point of probability was estimated, found that the highest magnitude of the probability increase occurred between 22 and 26 cm of scrotal circumference (40%), after that, magnitude of the probability between scrotal circumference adjacent measures decreased. Inflection point of the probability of reaching puberty occurred between 300 and 500 days of age (9%), after that age, magnitude of probability decreased.

Lunstra et al. (1978) reported that scrotal circumference, more than age and weight, is a good predictor of age at puberty in bulls, due to that, bulls of different European breeds and crossed non showed significant differences in scrotal circumference at puberty (ranking from 25,9 to 30,1 cm), even showing differences in the age and weight at puberty.

Thereby, results obtained in the present work are in agreement with results reported by Lunstra et al. (1978), and indicated that in zebu breeds, scrotal circumference from young bulls can be used for the identification of animals with early testicular growth, that is genetic correlated with age at puberty (Chapter 2). Thereby, scrotal circumference measure at early ages could be used as criterion selection for early sexual development bulls. Therefore, bulls that non reaches suitable parameters of testis development according to the respective breed in predetermined age, could be castrate and sell, minimizing the cost of maintenance and avoided entering in performance tests (Barth and Omiski, 2000).

According to Ferraz (2007), selection of bulls by scrotal circumference as sexual precocity indicator is a selection criterion and no an objective of selection, because the final objective will be a decrease of age at puberty

and increase of early pregnancy in heifers. Results obtained in the present work indicated that animals with larger scrotal circumference growth rate have higher probability of reaches puberty at early ages. In the same way, scrotal circumference can be used to indirect selection of early sexual development heifers, and this is due to the existence of a genetic correlation

between these traits (Boligon et al., 2007). Besides that, bulls with higher scrotal circumference begin to produce semen of high quality at early ages, and this could permit that these animals start the reproductive life more precocity, decreasing with this the generation interval (Barth e Omiski, 2000).

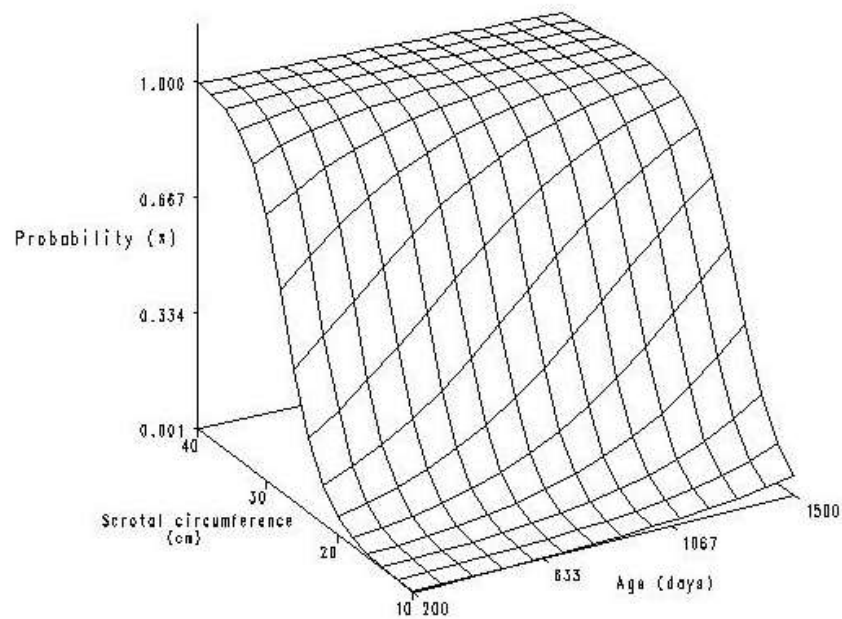


Figure 3. Probability of reaching puberty in function of age and scrotal circumference in Guzerat bulls

According to the figure 3, the interaction between scrotal circumference growth and age influenced the increase of the probability of reaching puberty in Guzerat bulls. Scrotal circumference was better predictor of puberty that age; however, these two variables act jointly, and for chose a selection criterion of sexual precocity is necessary know the variation of a trait in function of the other. In figure 3 can be observed that the probability of a animal reaching puberty before 20 cm of scrotal circumference is low, therefore, which the advance of age this probability increase (low magnitude), independent of the scrotal circumference have stayed less to 20 cm, in this

case, the most probable could be that the animal reaches puberty near or after 1000 days of age. However, from 22 cm of scrotal circumference is possible see how the highest influence over increase of probability of reach puberty is due to the increase of scrotal circumference; that is, even the animal be young, to each increase of a scrotal circumference centimeter, probability of reaching puberty increase. Nevertheless, magnitude of the increase of probability reaching puberty was higher with the increase of the age.

At 28 cm of scrotal circumference 84% of Guzerat bulls reached puberty. In this way, if the objective of scrotal circumference selection

is improve sexual precocity of bulls, the minor size of the scrotal circumference that is the best indicative of the pubertal stage of young bulls could be used as criterion selection.

It can therefore be concluded that probability of Guzerat bulls reaching puberty will be better

estimated having a reference of selection animal with scrotal circumference between 22 and 24 cm. Thereby, animals that reaches faster this scrotal circumference size, could be selected as sexual precocity animals with more probability of success.

## CHAPTER 4

### ASSOCIATION BETWEEN SCROTAL CIRCUMFERENCE GROWTH CURVE PARAMETERS AND AGE AT PUBERTY IN GUZERAT BULLS RAISED UNDER GRAZING CONDITIONS

#### Abstract

The aim of this work was evaluate the importance of the scrotal circumference parameters estimated by the Logistic model on the testis development, and evaluate the principal correlation between these parameters and age at puberty in Guzerat bulls raised under grazing conditions. The trait that best explained part of the phenotypic variation in the scrotal circumference and age at puberty between bulls into the first principal component was the k parameter and parameter A into the second principal component. The first principal component indicated that the major axis to describe variation among all the Guzerat bulls was associated with scrotal circumference growth rate. Parameter k had negative and low correlation with age at puberty; parameter k was positive and low correlated with scrotal circumference at 650 days of age. These results indicate that animals with higher scrotal circumference at early ages have early sexual development. The highest correlation found in the present work with the age at puberty was with the scrotal circumference at 650 days of age, indicating that Guzerat bulls with greater scrotal circumference at 650 days of age are younger at puberty.

#### Introduction

It is important to understand all the factors influencing the reproductive performance of bulls, one such factor are the testicular size, with scrotal circumference being the most common measurement of size (Baker and Kropp, 1991). Records of a growth trait when collected for the same animal from birth to maturity yield a growth curve, usually with sigmoid shape, and this records can be fitted by the used of nonlinear models (Fitzhugh, 1976). Parameter k of nonlinear models represents the velocity with the animal reach the adult testis size (parameter A), thereby, highest values of k indicate that the animal would reach fast the scrotal circumference at maturity (Martin Nieto et al., 2006).

According to Quirino et al. (1999) the point when the scrotal circumference growth rate

is maximal (auto-accelerate) would be associated with rapid proliferation of testis parenchyma, which suggested the beginning of the pubertal period. In sexual precocity Nellore bulls, the growth of the scrotal circumference is faster after 400 days of age and is maintained constant after puberty (Brito et al., 2004). This suggested that the age at puberty is influenced by the scrotal circumference growth rate.

Scrotal circumference is a trait with moderate to high heritability (Bergmann et al., 1997; Boligon et al., 2010; Silva et al., 2011; Loaiza-Echeverri et al., 2013(b)), and favorably correlated with growth traits like weaning weight and yearling weight (Boligon et al., 2010), testicular volume (Silva et al., 2011; Loaiza-Echeverri et al., 2013(b)), seminal quality (Bourdon and Brinks, 1986; Kastelick et al., 2001) and

female sexual precocity (Martínez-Velázquez et al., 2003; Boligon et al., 2007).

Some researchers recommended the use of the scrotal circumference measure at early ages (in general between 365 and 550 days) as selection criterion (Boligon et al., 2010; Silva et al., 2011). Few research reported genetic correlation between scrotal circumference measures at different ages. Alencar et al. (1993), Yokoo et al. (2007) and Boligon et al. (2010) reported positive and strong correlation between scrotal circumference measures, with greater values between adjacent measures, and with a decrease of the correlations with the increase of the interval between ages. However, the highest ages of the bulls evaluated in these works were 550 days and two years, respectively.

In zebu bulls raised under grazing conditions the sexual maturity initiated at 24 months of age (defined as the moment when the seminal parameters reached the minimum quality standards, >30% total sperm defects, as recommended by the Colégio Brasileiro de Reprodução Animal); nevertheless, after 30 months of age the number of bulls that reached sexual maturity is approximately 50% (Dias et al., 2007). According to Quirino et al. (1999), scrotal circumference still growth after 30 months of age and reach the plateau only after 40 months of age.

There is a delay in the literature in reporting the correlation between scrotal circumference growth rate (parameter  $k$  of nonlinear models) with the age at puberty, and between these traits with the scrotal circumference at maturity (parameter  $A$  of nonlinear model). Due to that, the aim of this work was evaluate the importance of the influenced of that factors on the testis

development, and evaluate the principal correlation between parameters of the Logistic nonlinear model, scrotal circumference at 650 days of age and age at puberty in Guzerat bulls raised under grazing conditions.

## Material and methods

### *Animals and Data*

This study was conducted on three farms located in Brasilândia de Minas (17° 00' 36" South and 46° 00' 32" West), Carlos Chagas (17°41'30" South and 40°45'15" West), and Unai (16°21'43" South and 46°54'09" West), in the state of Minas Gerais, Brazil. The climate classification in these regions is Aw (tropical rainy climate, Köppen classification), with average temperature of 18 °C in the coldest month. Dry season coincides with fall and winter, principally, and these seasons go from April to September. Spring and summer go from October to March. November, December, January and February concentrated approximately 60% of the total yearly rainfall. During the nursing period and after weaning (~ 7 mo old), all Guzerat males were raised under grazing conditions on palisade grass (*Brachiaria brizantha* Stapf cv. Marandu) and bluestem (*Andropogon gayanus* Kunth cv. Planaltina) in the savannah region with water and mineral salt *ad libitum*. During the dry season the animals were supplemented with salt-type protein or roughage.

Scrotal circumference was measured in the region of the greatest diameter of the testis and covered two gonads positioned symmetrically side by side, leaving the skin of the scrotum distended, the measurements

were obtained at intervals of approximately 3 months.

Semen collection by electro-ejaculation was attempted in the animals when they reached 20 cm of SC. Twenty centimeters was the threshold SC below which sperm cell collection was always unsuccessful (Torres-Júnior and Henry, 2005). The interval between semen collections was approximately 3 months, and onset of puberty was considered to be the age when the first mobile spermatozoa were observed in the ejaculate (Garcia et al., 1987; Torres-Júnior and Henry, 2005). When mobile spermatozoa were found 3 months after an unsuccessful attempt, onset of puberty was estimated by interpolation (half the time between the unsuccessful and successful semen collection).

Before the analysis the data base was edited. Animals that didn't have measurements of scrotal circumference before and after puberty were deleted and only animals with 5 or more records of scrotal circumference were maintained in the data base.

### **The model**

To estimate the growth curve parameters was used the Logistic nonlinear model,  $SC_{it} = A_i / [1 + B_i \exp(-kt)]$ , where  $SC_{it}$  is the scrotal circumference (SC) to  $t$  days of age of animal  $i$ ,  $A_i$  is the estimated SC at maturity of animal  $i$ ,  $B_i$  indicates the proportion of the asymptotic mature testis size to be obtained after birth of animal  $i$ , established by the initial value of SC and  $t$ ;  $k$  is a maturing index, establishing the earliness with which SC approaches  $A$ . The Gauss-Newton iterative method from Non Linear Regression (NLIN) of the SAS System (SAS Institute, 2001) was used to

estimate the parameters of the nonlinear model for each animal.

Estimates of scrotal circumference growth curve parameters were obtained based on 5811 SC-age records from 636 Guzerat bulls born between 2001 and 2009 using the Logistic nonlinear model. After this, animals where the convergence was not observed were deleted.

The general statistical model used in the GLM procedure

was:  $y_{ijk} = \mu + CG_j + M_k + \varepsilon_{ijk}$ , where  $y_{ijk}$  is the  $A$  or  $k$  parameter, scrotal circumference at 650 days of age or age at puberty of animal  $i$ ;  $\mu$  is the overall mean;  $CG_j$  is the fixed effect of contemporaneous group,  $j = 13$ ;  $M_k$  is the fixed effect of the month of birth,  $l = 12$ ;  $\varepsilon_{ijk}$  is the residual error associated with each information. The correlation between the dependent variables was estimated by the used of the MANOVA procedure of the SAS System (SAS Institute, 2001; SAS Institute, Cary, NC, USA). Scrotal circumference at 650 days of age was estimated by the nonlinear Logistic model adjusted for this specific age.

Principal components analysis was applied to scrotal circumference growth curve parameters ( $A$  and  $k$ ), scrotal circumference at 650 days of age and age at puberty records by the PRINTCOMP procedure of the SAS System (SAS Institute, 2001). In this procedure was utilized the correlation matrix and not the covariation matrix, because the records were measurements in different scales. The principal components technique consists in transforming a set of variables  $X_1, X_2, \dots, X_p$  in a new set of variables  $Y_1, Y_2, \dots, Y_p$  with the following features: each principal component ( $Y_i$ ) is a linear

combination of the standardized variables ( $X_i$ ). The first principal component had the maximum variance between all X combinations. The second principal component is not correlated with the first, and had the second maximum variance. Each principal component had p eigenvalues when  $\lambda_1 \geq \lambda_2 \geq \dots \geq \lambda_p$  (Souza, 2011).

## Results and Discussion

Means of the age at puberty, scrotal circumference at 650 days of age and the estimates of the scrotal circumference growth curve parameters are shown in the Table 1. The age at puberty reported here was higher than that reported by other authors for Guzerat bulls ( $540 \pm 60$  days,  $564 \pm 63$  days and  $588 \pm 117$  days; Troconiz et al., 1991; Perez et al., 2012 and Torres and Henry, 2005, respectively). This could be due to that the puberty records used in the present study are from bulls of different herds, and environments and genetic factors could be influencing the sexual development.

On the other hand, the scrotal circumference estimated at 650 days of age (Table 1) is close to the scrotal circumference at puberty reported by the same authors cited anteriorly:  $25.6 \pm 2.2$  cm,  $24.2 \pm 2.4$  cm and  $22.8 \pm 2.9$  cm, respectively; and this could be indicated that Guzerat bulls reach the puberty with a scrotal circumference between 22 and 25 cm approximately.

The mean of the A parameter estimated in the present work was little higher than mean of the A parameter reported for Guzerat bulls ( $35.96 \pm 0.2$  cm) that were part of the same data base used in the present work (Chapter 1), Nellore bulls ( $36.9 \pm 0.5$  cm) using the same nonlinear model (Quirino et

al., 1999), Retinto bulls (38.49 cm) using a similar Logistic model (Delgado et al., 2000) and that values reported for Canchim bulls raised under grazing conditions ( $35.5 \pm 0.10$ ) using the same nonlinear model (Martin Nieto et al., 2006). The high value of parameter A, and the high standard deviation estimated for this parameter could be due to the fact that in the present work we estimated the parameters of the Logistic model for each bull, and the number of scrotal circumference records of each bull ranged from 5 to 25. According to Toral (2008), the number and interval of the measurements of the any trait influenced the estimates of the parameters A and k of the nonlinear models.

On the other hand, the mean of the k parameter reported here was smaller than the value reported by Quirino et al. (1999) and similar to the value reported by Neves et al. (2011) for Nellore bulls. Neves et al. (2011) reported differences in the scrotal circumference growth rates between Nellore bulls of the same herd; and according to Vale Filho et al. (2001), differences of growth rates were associated with the age at first release of spermatozoon in the seminal plasma. Thereby, the results reported here could be due to differences in the scrotal circumference growth potential between the breeds, environmental factors and the number of records by animal, as seem previously (Toral, 2008).



Table 1. Means of age at puberty, scrotal circumference at 650 days of age and scrotal circumference growth curve parameters estimates by the Logistic model for Guzerat bulls.

Variables	N	Mean	SD	Min.	Max.
Age at puberty	504	627.49	140.14	363	1100
SC650	504	24.36	3.38	14.50	33.86
A	504	40.80	10.19	28.44	85.69
B	504	5.63	4.52	1.06	59.85
k	504	0.0033	0.0012	0.0011	0.0076

SC650 = scrotal circumference at 650 days of age; A = scrotal circumference at maturity estimated by the Logistic model; B = scrotal circumference at birth estimated by the Logistic model; k = maturing index estimated by the Logistic model.

Among the fixed effects studied, the contemporary group and month of birth influenced the parameters of the Logistic model, as well as the scrotal circumference at 650 days of age and age at puberty ( $P < 0.001$ ).

Martin Nieto et al. (2006) estimated the parameters of scrotal circumference growth curve for Canchim bulls raised in different breeding systems and show that animals in confined conditions had higher scrotal circumference at maturity than animals raised under grazing conditions. In Chapter 1 we reported that Guzerat bulls born in the dry season had the greatest values of A parameter of the scrotal circumference growth curve and this could be due to the absence of health problems in the birth season and weaning at more favorably times.

Age at puberty is associated with luteinizing hormone secretion during the period of early gonadotropin rise (Evans et al., 1995), and

was observed that bulls with medium or high nutrition management during calthood and peripubertal period had greater frequency and total secretion of LH in the early gonadotropin rise, were younger at puberty and had greater scrotal circumference at 70 weeks of age that bulls in low nutrition management (Brito et al., 2007 (a); Brito et al., 2007 (b)); these results indicating that management, specially the nutritional factor influenced the sexual development through effects on the GnRH pulse generator in the hypothalamus and effects on the testes, reflected in the scrotal circumference size and age at puberty.

In table 2 are shown the simple correlation between the variables. Correlation between parameter A and age at puberty was non-significant. Parameter k estimated by a nonlinear model had negative and low correlation with age at puberty; this correlation means that animals with high values of scrotal circumference growth rate can be young at puberty. On the other hand, parameter k was positive and low correlated with scrotal circumference at 650 days of age, indicating that animals with high values of scrotal circumference growth rate could have greater scrotal circumference size at 650 days of age that animals with lesser scrotal circumference growth rate. The negative and strong correlation between k and A parameters is indicating that Guzerat bulls with high scrotal circumference growth rate have lower scrotal circumference size at maturity that those bulls with low values of scrotal circumference growth rates. A similar example to the simple correlation estimated here can be observed in the distribution pattern shown in figure 1.

Table 2. Assessments of the coefficient of phenotypic correlation between scrotal circumference traits and age at puberty.

Traits	Age at puberty	SC650	A	k
Age at puberty	1.000			
SC650	-0.614 < 0.0001	1.000		
A	0.181 < 0.0001	-0.065 0.1516	1.000	
k	-0.287 < 0.0001	0.350 < 0.0001	-0.555 < 0.0001	1.000

SC650= scrotal circumference at 650 day of age estimated by the Logistic model; A = scrotal circumference at maturity estimated by the Logistic model; k = maturing index estimated by the Logistic model.

According to Chase et al. (2001) crossed bulls of Tuli x Angus and Senepol x Angus breeds presented greater scrotal circumference growth rate and were younger at the time of presence of the first spermatozoa in an ejaculate than Brahman x Angus bulls. Similarly, Brahman x Angus bulls were older at 50 and 500 x 10<sup>6</sup> sperm per ejaculate; however, these animals had greater scrotal circumference at this time than the others breeds.

These results confirmed the correlation reached between parameter k and age at puberty, indicating that animals with higher scrotal circumference at early ages have

early sexual development; similar results were reported by other authors (Lunstra et al., 2003; Brito et al., 2004). Nevertheless, the results presented by Chase et al. (2001) also could be explain the correlation found between parameter A and k reported in the present work, and could be indicating that animals with lower scrotal circumference growth rate at early ages have greater gains in scrotal circumference size at late ages, and that these animals can have the same or greater scrotal circumference at maturity than animals that had higher scrotal circumference growth rate at early ages, as reported by Lunstra et al. (2003).

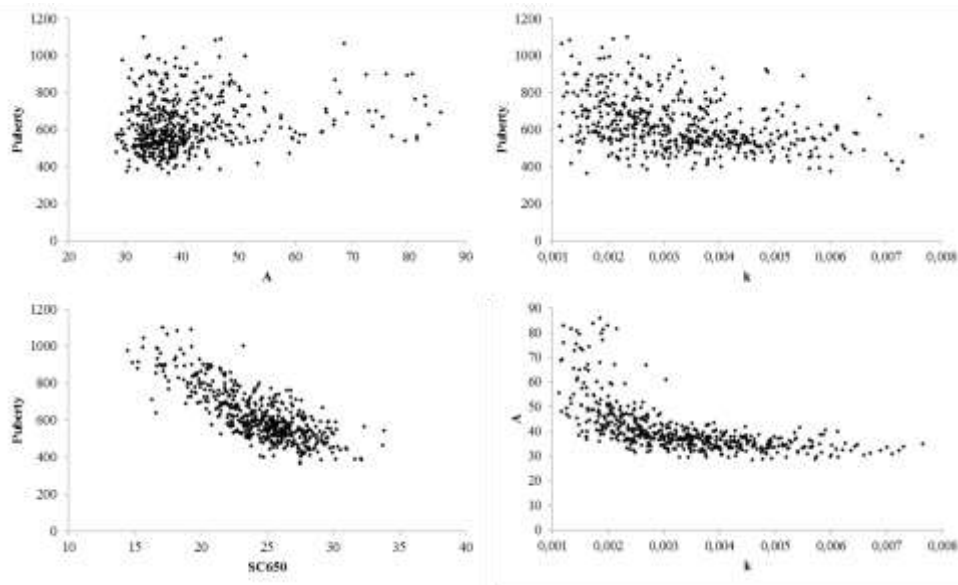


Figure 1. Distribution pattern of the age at puberty data in function of the parameter A, k and scrotal circumference at 650 days of age; and parameters A in function of the parameter k estimates by the Logistic model.

The results obtained for the principal components, eigenvalues and percentage of total variance explicated by each components are shown in Table 3. Principal components are linear combinations of a set of covariates, and the objective of this procedure is provides the principal components that describe the maximum variation among a set of observation (Baker et al., 1988).

Table 3. Eigenvalues and percentage of the variation explained by the principal components for scrotal circumference traits and age at puberty.

Principal component	Eigenvalues	Percentage (%)	Cumulative Percentage (%)
1	2.041	51	51
2	1.167	29	80
3	0.481	12	92
4	0.308	07	100

The first principal component explained 51% of the variation in the scrotal circumference growth and the age at puberty, and combining the first and the second principal component accounted 80% of the total variation. The remaining two components combined accounted for only 20% of the total variation (Table 3). According to this result, any linear combinations of the original variables could explain more than the first component the phenotypic variation.

In the present work, the trait that best explain part of the phenotypic variation in the scrotal circumference and age at puberty between bulls into the first principal component (PC1) was the k parameter, with great involvement in the 51% of the variation explained by this principal component (Table 3). Parameter A was the trait that best explain part of the phenotypic

variation into the second principal component (PC2).

According to Baker et al. (1988), total variation in pubertal characteristics (age, weight and height at puberty) of heifers and bulls was described by the first component (70%), when the importance of the size and scale and the minimal influence of age in describing the principal axis of variation among animals is indicative of the physiological relationship among the characters. Thus, these authors reported the high interrelationship of size, growth and puberty, indicating that measures of skeletal are less susceptible to environmental variation than weight, and that this trait could be used in selection programs. However, in this work they not evaluated the scrotal circumference influence on age at puberty.

The correlation between the principal component and the original variables in the vector are in Table 4. Large negative correlation existed between the first component and age at puberty and between the first component and parameter A (scrotal circumference at maturity). Large positive correlation existed between the first principal component with parameter k

(maturing index) and with scrotal circumference at 650 days of age.

Therefore, the first principal component indicated that the major axis to describe variation among all the Guzerat bulls was associated with scrotal circumference growth rate. Larger values of scrotal circumference growth rate were associated with younger pubertal ages and larger values of scrotal circumference at 650 days. However, the negative correlation with parameter A indicated that larger size of scrotal circumference at early ages and precocious puberty were associated with smaller size of scrotal circumference at maturity.

The second principal component explained 29% of the total variation in the scrotal circumference growth and age at puberty, and had moderate negative coefficients for age at puberty and parameter k. This principal component was largest positive correlated with parameter A, and indicated that animals with largest scrotal circumference at maturity had lower scrotal circumference growth rate and relative minor scrotal circumference at 650 days and were relative older at puberty.

Table 4. Assessments of the eigenvectors associated to the principal components of variation for scrotal circumference traits and age at puberty and correlation among these traits and the principal components.

Traits	Weighting coefficient				Correlation			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
Age at puberty	-0.524	-0.427	0.576	0.458	-0.749	-0.461	0.400	0.254
SC650	0.516	0.491	0.352	0.606	0.738	0.530	0.244	0.336
A	-0.410	0.649	0.460	-0.444	-0.586	0.701	0.319	-0.246
k	0.537	-0.392	0.575	-0.474	0.768	-0.424	0.399	-0.263

PC = principal components; SC650 = scrotal circumference at 650 days of age; A = scrotal circumference at maturity estimated by the Logistic model; k = maturing index estimated by the Logistic model.

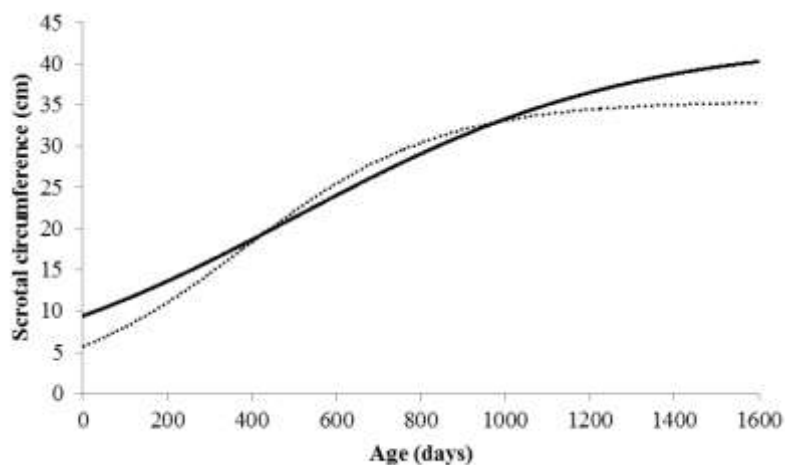


Figure 2. Scrotal circumference growth curve of two Guzerat bulls with high A parameter value and low k parameter value ( $A = 42,768,212.5$ ;  $k = 0,00252108$ ; line) and low A parameter value and high k parameter value ( $A = 35,444,132.7$ ;  $k = 0,00431739$ ; points) estimated by the Logistic model.

Low scrotal circumference growth rate in some heat-adapted breeds is associated with delay in reach puberty; because of that, are necessary procedures to emphasize growth rate and early sexual development for heat-adapted in genetic improvement programs (Lunstra et al., 2003). Nevertheless, the implication of the scrotal circumference growth rate in the scrotal circumference size at maturity need more study.

In agreement with the results reported by Lunstra et al. (2003), in the present work found that animals with low values of k parameter were late at maturity. Scrotal circumference growth curve of two Guzerat

bulls were represented in Figure 2, where one bull reach puberty at 590 (high value of k and low value of A) and the other bull at 772 days of age (high value of A and low value of k). This result indicates that animals with fast testicular growth could be selected as animals of precocious sexual development.

The highest correlation found in the present work with the age at puberty was with the SC650 trait, and this could be indicated that for Guzerat bulls raised under grazing conditions, the scrotal circumference measure at this age would be more suitable for use as criterion selection for se

xual precocity than the scrotal circumference growth rate (parameter k) estimated by a nonlinear model. The highest genetic correlation found between scrotal circumference and age at puberty in Guzerat bulls was obtained at 650 days of age (Chapter 2); thereby, these results indicate the suitability of the use of this trait as criterion selection for sexual precocity. In addition to that, scrotal circumference is an easily trait to be measured, while for the estimate of the k parameter are needed a series of scrotal circumference records during the life of the animals.

According to this results could be expected that animals with more scrotal circumference growth rate at early ages have higher scrotal circumference at 650 days and be younger at puberty. However, to estimate the scrotal circumference growth rate a sequence of scrotal circumference measures must be taken, and this could be hardly executed in large herds.

We do not reach reports of heritabilities of parameters of the scrotal circumference growth curve estimated with the nonlinear models. On the other hand, heritability for scrotal circumference of Guzerat bulls at 650 days of age was estimated in 0.66 and for age at puberty from 0.46 to 0.55; at 970 days of age the heritability of scrotal circumference was 0.61, and this could be indicate that heritabilities for scrotal circumference decrease with the age (Loaiza-Echeverri et al., 2013(b)). According to these results, and the results exposed in the present work, could be expected that selection of animal by the greater scrotal circumference at 650 days of age are younger at puberty.

The principal finding exposed through the results was the high correlation between the scrotal circumference growth rates with greater scrotal circumference at early ages and early

age at puberty; furthermore, animals with higher scrotal circumference growth rate at early ages could have minor scrotal circumference at maturity. On the other hand, due to the favorably correlation of the scrotal circumference at 650 days of age with the age at puberty, and the easy measure of this trait, could be proposed the SC650 as criterion selection for improvement the sexual precocity of Guzerat bulls raised under grazing conditions.

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## IMPLICATIONS

- The parameters A and k of non-linear models can be used to analyze the scrotal circumference growth of the bulls; however, some models feature low setting, possibly because of the small amount of data available at the early life of animals and in adulthood, or the high interval between data collections. Because of this, it is necessary to evaluate different non-linear models to identify the best fit to the available data. The present study found that the Logistic model showed the best fit to scrotal circumference data.

- Environmental factors, especially the season of birth, influence the growth rate and the scrotal circumference of the adult animal, as result, management strategies (such as food, definition of the breeding season and calves birth) and selection could be used in order to modify the scrotal circumference growth curve.

- The scrotal circumference growth rate (estimated by Logistic model, parameter k) is positively correlated with age at puberty and scrotal circumference at 650 days of age in Guzerá bulls, thus, the selection of animals with larger testicular size in early ages could help to improve the sexual precocity of the bulls. However, the negative correlation between the growth rate (k parameter) and scrotal circumference of the adult animal (parameter A), although low, could indicate that animals with higher growth rates at younger ages reach a smaller scrotal circumference when adults; while animals of lower growth rates at early ages reach larger scrotal circumference at adults.

Thus, it is necessary to evaluate the consequences of selecting young animals for sexual precocity on sperm production of the bulls in adulthood, which was an important

factor in extensive farming systems, where the main form of mating is done by natural mating.

- The principal component analysis demonstrates the importance of growth rate of the scrotal circumference in relation to the variation in the age at puberty and size of scrotal circumference in Guzerat bulls, and indicates a physiological relationship existing between these characteristics.

- This paper illustrates the interrelationship of the scrotal circumference growth rate, scrotal circumference of young animal and age at puberty, and indicates the possibility of using scrotal circumference measured at young ages, in selection programs to improve sexual precocity.

- The scrotal circumference can be used as a marker to predict the age at puberty in bulls; however, there is variability in age at puberty among bulls of different breeds and within breeds; therefore, it is important to define the age in which each breed can be evaluate by the scrotal circumference size as way to predict the pubertal stage of animals. According to the results of this study, the probability that Guzerat bulls reach puberty increases with the increase of the scrotal circumference size and at 24 cm scrotal circumference is possible that 66% of the bulls have reached puberty. Similarly, these results indicate that animals with higher scrotal circumference growth rates achieve faster 24 cm, and can be selected as early sexual development animals. These results could be used for further experiments planning that needs established the best age to selection animals for age at puberty.

- This paper illustrates the interrelationship of the growth rate of scrotal circumference, scrotal circumference of young animal and puberty, and indicates the possibility of using scrotal

circumference, measured at young ages, in selection programs to improve sexual precocity.

It was found in this study that scrotal circumference, testicular volume, weight and age at puberty in Guzerat bulls have moderate to high heritability, and scrotal circumference measured at 650 days of age, is the characteristic with highest heritability. Between all traits, scrotal circumference was the trait with the highest genetic and phenotypic correlation with age at puberty, and the highest correlation was at 650 days of age. These results indicated that it is not necessary to use the testicular volume as a selection criterion for sexual precocity; this is a feature that requires several testicular measures and the use of a mathematical formula to be calculated, or which can lead to errors of precision.

- The selection for any of the traits evaluated in this work will produce favorable correlated response in age at puberty in Guzerat bulls; however, the greatest correlated response can be obtained when the selection is based on scrotal circumference measured at 650 days of age.

With the results presented in this work, we can indicate the scrotal circumference measured at 650 days of age as criterion selection to be used in Guzerat bulls to improve sexual precocity.

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