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SERIC CONCENTRATION OF REPRODUCTIVE HORMONES AND MINERALS IN PREPUBERTAL AND PUBERTAL POLLED NELLORE BULLS

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ABSTRACT

Nellore polled (*Bos indicus*) bulls are used to introduce characteristics of environmental resistance in meat-producing herds, however, the morphophysiological characteristics related to body structure and reproductive parameters are not well described. The aim of this study was to evaluate the scrotal circumference (SC), body weight (BW) the plasma concentration of LH, testosterone, triiodothyronine (T3), thyroxine (T4), copper and zinc of prepubertal and pubertal Nellore Pulled bulls under field conditions. Records of 2002 and 2003 of fifty-five Nellore pulled bulls between 12 and 24 months old of Boa Vista farm in São Paulo state, Brazil were used. The SC, BW and blood samples for hormonal and microminerals assay were evaluated monthly. The mean values of SC and BW were: 24.91 ± 2.81 cm, 304.35 ± 1.30 kg, respectively. The mean plasma concentrations of LH, testosterone, T3 and T4 were 0.25 ± 0.08 ng/dl^{-1}, 163.5 ± 95.70 ng/ml^{-1}, 1.31 ± 0.12 ng/dl^{-1} and 2.32 ± 0.1 $\mu g/dl^{-1}$, respectively. The average levels of Cu and Zn were 0.29 ± 0.1 mg/l^{-1} and 0.27 ± 0.03 mg/l^{-1}, respectively. Similarly, to other *B. indicus* breeds the SC, BW, LH, testosterone, T3 and T4 and the minerals Cu and zinc Zn showed age effect ($p<0.01$). Body weight and scrotal circumference are important in the estimation of sexual maturity and are dependent on testosterone and LH levels. The results of concentrations of Cu and Zn did not impair the development of Nellore polled bulls. The parameters analyzed in prepubertal and pubertal Nellore polled bulls are influenced by age and are similar to those in Bos indicus bulls in the field condiction.

KEYWORDS: *Bos indicus*, puberty, testicular biometry.

CONCENTRAÇÃO SÉRICAS DE HORMÔNIOS REPRODUTIVOS E DE MINERAIS EM TOUROS NELORE MOCHO PRÉPUBERE E PÚBERES

RESUMO

Touros Nelore mocho são utilizados para introduzir características de resistência ambiental em rebanhos de produção de carne, entretanto, as caracteristicas morfofisiológicas relacionadas a estrutura corporal e parâmetros reprodutivos são pouco elucidades. Objetivou-se avaliar a circumferência escrotal (CE), peso corporal (PC), a concentração plasmática de LH, testosterona, triiodotironina (T3), tiroxina (T4), cobre e zinco de touros Nelore mocho pré-púberes e púberes em condições de campo. Foram utilizados registros de 2002 e 2003 de touros Nelore mocho (n=55) da fazenda Boa Vista, São Paulo, Brasil. CE, PC e amostras de sangue foram coletadas mensalmente. Os valores médios de CE e PC foram: 24,91 ± 2,81 cm, 304,35 ± 1,30 kg, respectivamente. As concentrações plasmáticas médias de LH, testosterona, T3 e T4 foram 0,25 ± 0,08 ng/dl^{-1}, 163,5 ± 95,70 ng/ml-1, 1,31 ± 0,12 ng/dl-1 e 2,32 ± 0,1 µg/dl^{-1}, respectivamente. Os teores médios de Cu e Zn foram 0,29 ± 0,1 mg/l^{-1} e 0,27 ± 0,03 mg/l^{-1}, respectivamente. Da mesma forma, para outras raças de *B. indicus* o CE, PC, LH, testosterona, T3 e T4 e os minerais Cu e zinco Zn apresentaram efeito (p<0,01) da idade. O CE e o PC são mensurações importantes para estimar a maturidade sexual e dependem dos níveis de testosterona e LH. Os resultados das concentrações de Cu e Zn não prejudicaram o desenvolvimento dos touros Nelore mocho. Os parâmetros analisados em touros Nelore mochos pré-púberes e púberes são influenciados pela idade e são semelhantes aos de touros *Bos indicus* em condição a campo.

PALAVRAS-CHAVE: biometria testicular, *Bos indicus*, puberdade, touros.

INTRODUTION

Sexual precocity and sperm production capacity are important traits for a high performance of breeding bulls and such parameters are dependent on feeding, endocrine factors and testicular development (*MOURA* et al., 2002). The scrotal circumference (SC) is a quantitative parameter, which has high heritability and repeatability on progenie (MORAES *et al*., 2019; SCHMIDT *et al*., 2019), in addition it has a positive correlation with body weight (BW) and age at puberty, which in association with the assessment of physical and morphological characteristics, are the basis of reproductive selection (CHENOWETH, 2011). Furthermore, SC presents a progressive increase until puberty, followed by a slower growth until sexual maturity, being, therefore, an indicator of such (QUIRINO; BERGMANN, 1998). Furthermore, SC is directly associated to sperm production (VÁ*SQUEZ* et al., 2003). Simultaneously to SC, the pulsatile secretion of luteinizing hormone (LH) increases, which is related to onset of puberty. One of the most important functions of LH is to promote initial growth and development of bovine testes, as well as support the formation of seminiferous tubules and the differentiation of Leydig and Sertoli cells (*EVANS* et al., 1995).

Another important aspect to be considered is Testosterone production, which is mediated by stimulatory actions of LH, being also related to age at puberty, sexual development and maturity (MOURA; ERICKSON, 1997). Interestingly, a slight increase of testosterone concentration from 13 mounths of age onwards (*MOURA* et al., 2002), plays a essencial role in the maintenance of spermatogenesis (ZIRKIN, 1998), demonstrating, therefore, the activity of Leydig cells (ASSU*MPÇÃO* et al., 2013) an essential for puberty.

The puberty in bulls is confirmed with the production of a sperm sample containing at least 50 x10^6 sperm cells presenting a minimum of 10 % progressive motility, display oflibido is also a requirement *(WOLF* et al., 1965). After puberty, blood concentrations of GnRH and Testosterone increase alongside the body weight, co-promoting gonadal growth and marked modifications in sperm quality. With puberty, sperm progressive motility increases to 50 % and morphology will present a maximum of ~20 % defects, furthermore, 16-20 weeks after the onset of puberty, the sexual maturity is stablished when proper testosterone levels are reached as well as achieval of full sexual development (RAW*LINGS* et al., 2008).

Another fundamental factor are the Thyroid hormones, which besides being a regulator of growth, development, and metabolism (M*ULLUR* et al., 2014) is also related with the control of Sertoli cell proliferation and functional maturation *(ZHOU* et al., 2002), as well as Leydig cells differentiation *(ZHAO* et al., 2001) and, expression of androgen receptor (*FLOOD* et al., 2013). Interestingly, studies presented a cross-relationship between the thyroid hormones and hypothalamic-pituitary–testicular axis, in which Gonadotrophin Release Hormone (GnRH) affects thyroid stimulating hormone (TSH) release. Furthermore, Fernández *et al*. (2014) demonstrated an association between polimorphism of deiodinase, an enzyme responsible for the formation of TH, with age of puberty.

Lastly, microminerals are also important to reproduction development, contributing for increase in SC and testicular weight, which were positively associated with testicular development and function as well as reproductive performance in bulls (*KUMAR* et al., 2014; 2011). Other crucial function of such agents is related to antioxidant response, and integrity of acrosome and plasma membrane (ARANGASAMY *et al*., 2018). Moreover, bulls supplemented with trace minerals reached puberty 15 before when compared to animals that received sulfate minerals supplementation. (*GEARY* et al., 2019).

It’s important to indicate, that concerning puberty, none of the studies described above involved the Nellore polled breed. Therefore, the knowledge of the dynamic of reproductive and thyroid hormones as well as mineral plasmatic concentration is important to describe the main alterations during the puberty for the breed under field conditions. Considering the evidence on testicular development, the aim of this study was to perform detailed analyzes under field conditions of the SC, BW, plasma concentration of LH, testosterone, triiodothyronine (T3), thyroxine (T4) and plasma levels of copper (Cu) and zinc (Zn) from peri-pubertal age to sexual maturity in prepubertal and pubertal Nellore polled Bulls.

MATERIALS AND METHODS

Animals and feed management

Records of 2002 to 2003 of fifty-five Nellore polled bulls (*Bos indicus*) from Boa Vista farm in São Paulo state, Brazil were used. The bulls were kept under pasture (*Brachiaria brizantha*), mineral supplementation and water *ad libitum* from 12- 24 mo of age (Table 1). The bulls were selected based on the Breeding Program of the Brazilian Association of Zebu Breeders (PMGZ/ABCZ). All activities involved in the project were reviewed and approved by Institutional Animal Care and Use Committees at the UNESP-Botucatu. In addition, this work was performed in compliance with relevant guidelines and regulations regarding utilization of animals in research.

Scrotal circumference and body weight measurements

The Scrotal circumference (SC) and body weight (BW) were evaluated monthly, from 12 to 24 mo of age, in a farm in Indiana, São Paulo, latitude 22°07’04”, longitude 51°22’5” and altitude of 435,5m. The SC was measured by placing a tape (Walmur – São Paulo, Brasil) around the scrotum at the point of maximum diameter, the results were obtained in centimeters (COLÉGIO BRASILEIRO DE REPRODUÇÃO ANIMAL, 2013). The BW of each animals was obtained using an electronic scale (kg).

Blood samples

Blood sample were collected monthly (jugular vein), from 12 to 24 mo of age, samples were collected in vacutainer tubes (Vacutainer®, Labnew® Indústria e Comercio Ltda, Juiz de Fora, MG, Brazil) with EDTA and centrifuged at 1500 x *g*, for 15 min being immediately stored at -20°C until time of the assays. The plasma samples used for for hormone concentration assayas were processed in the Endocrinology laboratory, Department of Animal Reproduction and Veterinary Radiology, Faculty of Veterinary Medicine and Animal Science, FMVZ, Campus de Botucatu.

Hormones concentrations

Quantification of LH was performed by RIA with ^{125}I through the Iodo Gen® method (1,3,4,6 – tetrachlor 3, 6 – difenilglicuril Sigma Company®). The testosterone, T3 and T4 hormone quantification were performed by RIA using, respectively a scintillation counter (COBRA II 5010 Gamma Count) with Coat-A-Count® Total Testosterone (Diagnostic Products Corporation, Los Angeles, CA), Coat-A-Count® Total T_3 (Diagnostic Products Corporation, Los Angeles, CA) and Coat-A-Count® Total T_4 (Diagnostic Products Corporation, Los Angeles, CA), following manufacturer instructions. Intra and inter-assay CVs were <20 % for all hormones.

Copper and zinc concentrations

Copper and Zinc concentrations were measured through flame atomic absorption spectrometry (Thermo Jarrel Ash, Germany). Aliquots (0.5 ml) were removed from the samples, adding 3 ml of nitric acid [HNO_3, py, T = 65 % (m / m), p = 1.37 kg l^{-1}] [6] and 2 ml of concentrated hydrogen peroxide solution [H_2O_2, py, T = 32 %] [7]. Serum samples were diluted in deionized water. Different concentrations of trace elements were prepared for calibration of standard graphs and absorbances were read at 213.9 nm. Serum samples were run in triplicate.

Statistical analysis

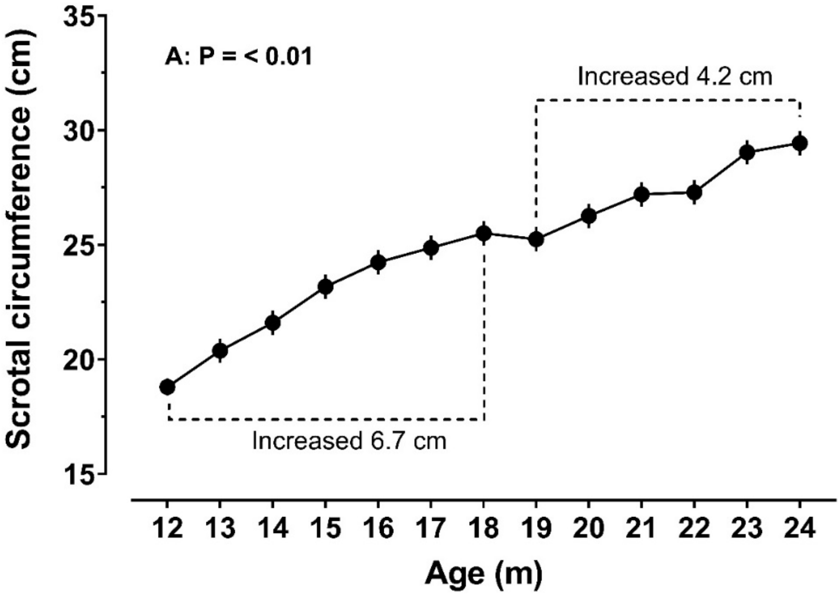
Statistical analyses were performed using the SAS (Version 9.4. Cary, NC, USA). Analyzes of SC, LH, testosterona, T3, T4, Cu and Zn were subjected to ANOVA with repeated meansured over the time (Proc Mixel), effect fixed of age was included in the model. Data are show with mean ± standard error of the mean (SEM). Differences were considered significant when p<0.05.

RESULTS

Scrotal circumference

An age effect was observed for SC (p<0.01). Additionally, the average observed in 55 bulls was 24.91 ± 0,28 cm, during the evaluation of the 12 mo of experiment. The scrotal circumference presented two distinct patterns of growth, a first increase (6.7 cm) occurred between 12 to 18 mo, while a second (4.2 cm) occurred between 19 to 24mo (Figure 1).

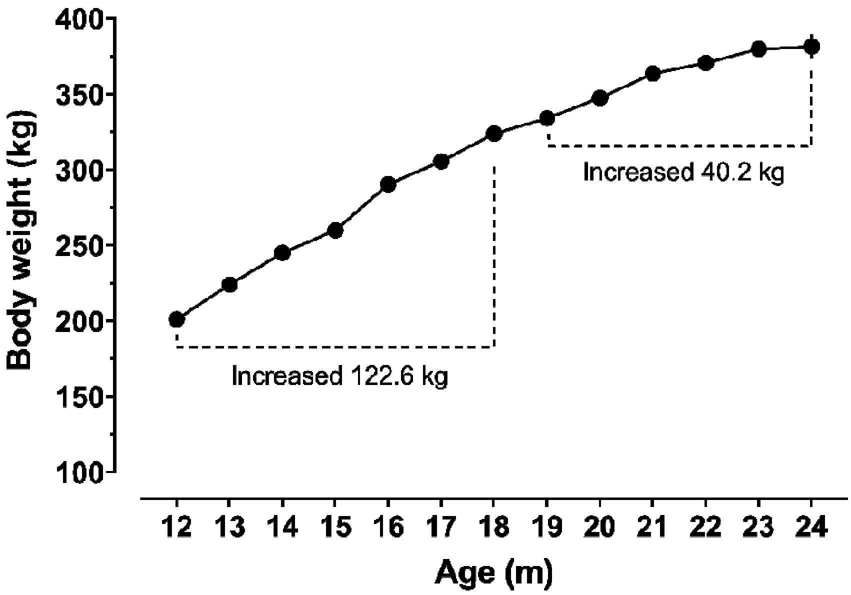
FIGURE 1. Scrotal circumference (mean ± SEM) of Nelore pulled bulls (n=55). Probability of age effect is shown (A). São Paulo, Brazil, 2003.



Body weight

An age effect was observed for Body weight (BW) ($p<0.01$). BW mean for the 55 bulls was 309.82 ± 1.30. Spiked body weight gain was observed between 12 to 18 mo old (122.6 kg), and then a more uniform growth was observed between 19 to 24 mo old (40.2 kg) (Figure 2).

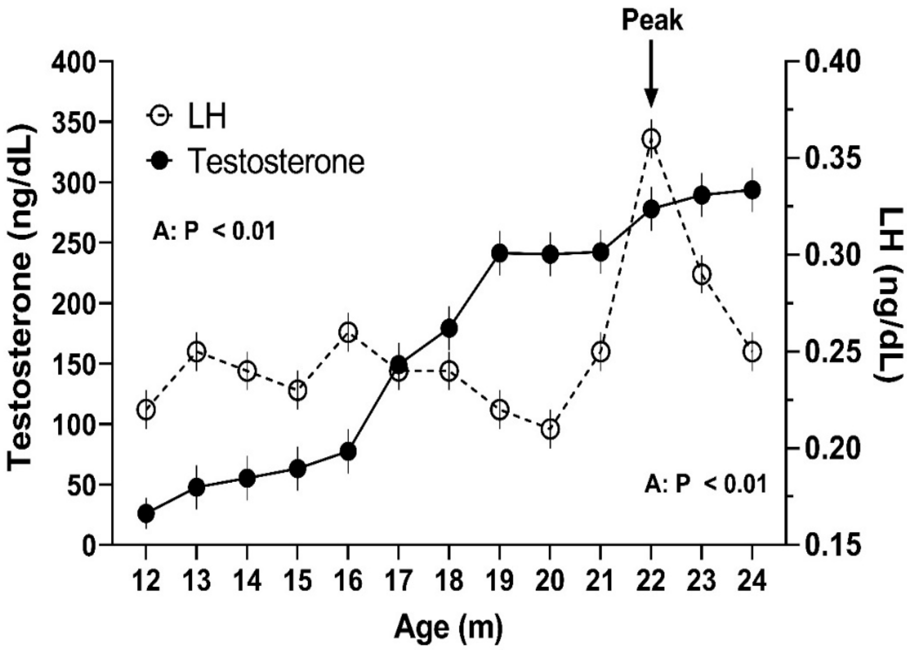
FIGURE 2. Body weight (mean ± SEM) of Nellore Pulled Bulls (n = 55) from 12 to 24 mo old. São Paulo, Brazil, 2003.



Hormone concentration (LH, testosterone, T³ and T⁴)

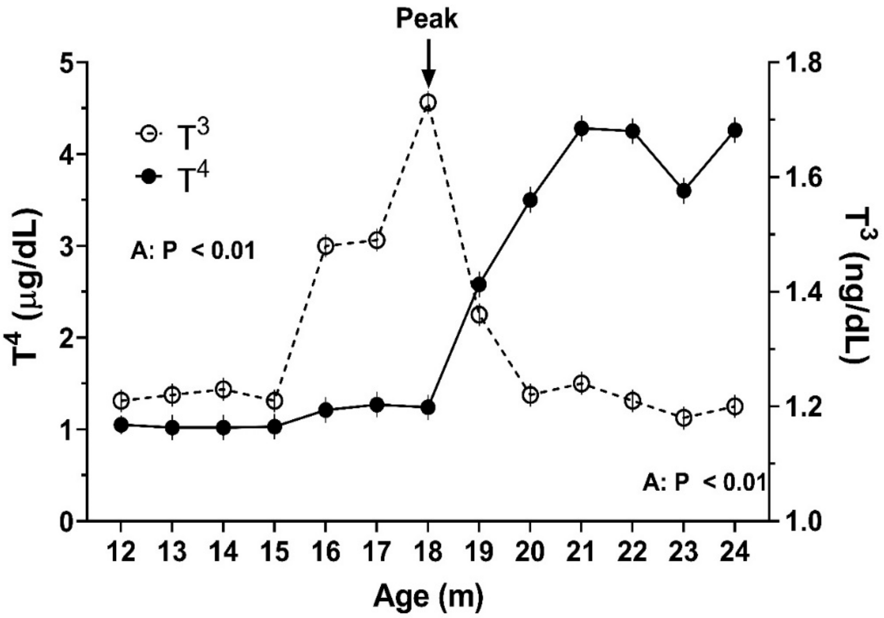
Plasmatic concentrations of luteinizing hormone (LH) and testosterone were significantly affected by age (p<0.01). The mean concentration was 0.25 ng/dl^{-1} and the peak of LH concentration was observed on 22 m of age (0.36 ng/dl^{-1}) (Figure 3). Testosterona plasmatic concentration peaked between 16 and 19 mo old (163.5 ng/dl-1). In addition, minimal increase was observed between 12 and 15 mo (51.4 ng/dl^{-1}) and 20 to 24 mo old (52 ng/dl^{-1}) (Figure 3).

FIGURE 3. Plasma concentrations (mean ± SEM) of LH (○) and Testosterone (●) of Nelore Polled Bulls (n = 55) from 12 to 24 mo old. (**A**) indicates the probability of age effect. São Paulo, Brazil, 2003.



Furthemore, plasmatic concentration T3 and T4 exibited a significant effect of age (p<0.01). The mean concentration of T3 was 1.31 ± 0.12 ng/dl^{-1} (Figure 4). A spike was observed between 15 and 18 mo old (0.5 µg/dl^{-1}), peaking at 18 mo old (1.7 µg/dl^{-1}) (Figure 4). In addition, the mean plasmatic concentration of T4 was 2.32 ± 0.10 µg/dl^{-1} (Figure 4). A major increase took place between 18 and 21 mo old (3.04 µg/dl^{-1}) and peaked at 21 mo old (4.28 µg/dl^{-1}).

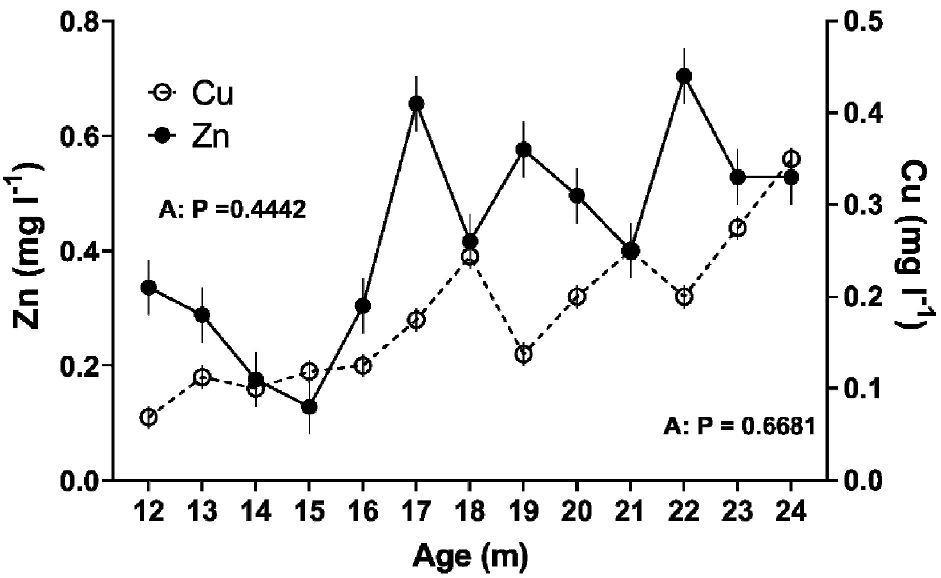
FIGURE 4. Plasma concentrations (mean ± SEM) of triiodothyronine (T3 ○) and thyroxine (T4 ●) in Nelore Polled Bulls (n = 55) from 12 to 24 mo old. (**A**) indicates the probability of age effect.



Cu and Zn plasmatic concentrations

No significant age effect was observed for Cu and Zn (p=0.6681 and p=0.4442, respectively). Additionally, mean concentrations were 0,29 ± 0.14 and 0.27 ± 0.03 mg/l^{-1} for Cu and Zn, respectively (Figure 5).

FIGURE 5. Plasma concentrations (mean ± SEM) of Copper (Cu ○) and Zinc (Zn ●) in Nellore Pulled Bulls (n = 55) from 12 to 24 mo old. (**A**) indicates the probability of the age effect.



DISCUSSION

This is the first study on Nellore polled breed which describes the variations of SC, body weight gain, serum concentrations of LH, testosterone, T^3, T^4, Cu and Zn in bulls under the extensive production system, from peri-puberty to sexual maturity.

Previous studies in Nellore Bulls demonstrated similar results for SC (ASSUMPÇÃO et al., 2013; MONTEIRO et al., 2011; MOURA et al., 2002; SEGUI et al., 2012; SILVEIRA et al., 2010). The major increase in SC was observed between 12 and 18 m of age with daily rate mean of SC (0.030 mcm). Similary results was observevd by Assumpção *et al*. (2013) and *Silva* et al. (2002), with 0.037 and 0.033 cm, respectively. This increased rate between 12 and 18 mo is probably related to the increase number of germ cells, volume of sertoli cells and diameter of seminiferous tubule (AMANN; WALKER, 1983; CURTIS; AMANN, 1981; KASTELIC, 2014). Furthermore, it's associated with the higher testosterone levels at 18 mo of age (*MOURA* et al., 2002), preceding the puberty and the first ejaculatory response (CURTIS; AMANN, 1981; *WOLF* et al., 1965). On the other hand, a slower groth rate in SC was observed between 18 and 24 m of age. The SC daily mean at 18 and 24 m of age in our study was inferior (0.023 vs 0.041 cm) compared to Assumpção *et al*. (2013). Furthermore, in this study, the bulls were fed in a dry period in a field conditions with mineral supplementation, different to Assumpção *et al*. (2013) which the bulls received a protein supplementation of 2 kg day^{-1}. Presumably the minor mean in the SC development after 18 m of age is related to nutritional management. The less increase observed between 18 and 24 months can be explanied by full development of testicular parenchyma (BARTH; OKO, 1989).

The body weight gain was accentuated between 12 and 18 m of age (122.6 kg) and similary to other *Bos indicus* breeds, such as Brahman (125.8 kg) (C*HACUR* et al., 2018). Although the lower increase between 18 and 24m of age (57.5 kg), the nutritional management and body growth are important factors for testicular development (ASSU*MPÇÃO* et al., 2013). Chacur *et al*. (2018) observed a significant correlation on body and reproductive tract morphometry in Brahman breed, which can be use in the selection of young bulls in field conditions. The weight gain in the puberty of male beed breed cattle is associated with the testosterona production (REDDY *et al*., 2015) Early nutritional management increases the testosterone concentration and it was associated with circulating levels of Insulin Growth Factor -1 (IGF-1) in bulls. IGF-1 is an important mediator of cellular growth, increasing the cellular metabolism and the proliferation of Leydig cells (*BRITO* et al., 2007).

The peak of plasma concentrations of testosterone occurred between 16 and 19 m of age, the results were increased by ~300 percent followed by a slight increase until 24 m of age. The concentrations levels of testosterone vary accorging to age (Amann & Schanbacher, 1983), reduced value before 15 m of age, gradual increase from 16 m of age and maximum values between 17 to 18 m of age in Nellore Bulls (*MOURA* et al., 2002). In addition, testosterone concentrations are considered as markers of the onset of puberty, since it indicates activity of Leydig cells, responsible for the production of testosterone. Furthermore, the plasma concentrations of testosterone are correlated with the increase in number of sperm and a decrease in sperm abnormalities (EVANS *et al*., 1995).

Classicaly, the first LH peak is observed before of testicular development and beginning of spermatogenesis (RAW*LINGS* et al., 1978; AMANN; WALKER, 1983). Therefore, LH controls the proliferation and differentiation of Leydig and Sertoli cells from postnatal phase onwards, being fundamental for the development of germ cells (WALKER, 2003; *BRITO* et al., 2007;). Age at puberty in *Bos taurus* bulls was related with frequency and magnitude of LH pulses after GnRH treatment according to observations by Evans *et al*. (1995).

This is the first report evaluating plasma THs concentrations in Nellore polled bulls from 12 to 24 months. In this study, the results of T3 (1.31 ± 0.12 ng dl^{-1}) and T (2.32 ± 0.10 ng dl^{-1}) were different as described by Rennó *et al*. (2019; 1.77 ng ml^{-1} for T³ and 9.65 µg dl^{-1} ou 965,000 ng dl^{-1}) in finishing Nellore Bulls (*B. indicus*) in a feedlot perspective. In our study, plasmatic T3 concentration remained constant until 15 months of age with an increase at maximum concentration with 18 months of age, period related to spermatogenic stabilization phase (OHASHI *et al*., 2007). These observations are possibly due the influence of THs hormones on proliferation, differentiation *(ZHAO* et al., 2001; 2002) and maturation of Sertoli cells (COOKE *et al*., 1984). In mice and rats, the length of the period of Sertoli cell proliferation during prepubertal development is set by the emergence of thyroid hormone signaling, otherwise Marshall & Plant (1996) suggests that in Rhesus monkeys there is a surge in setoli cell proliferation during puberty. Futhermore, the delay in sexual development is related with hypothyroidism, due the difficulty in differentiation of Leydig and Sertoli cells (DUARTE-GUT*ERMAN* et al., 2014; HOLSBERGER; COOKE, 2005). Although, our studies did not focus on correlation of Sertoli Cells and TH hormone, presumably, we suggest that the concentrations levels of THs hormones show signifficant pattern of levels that can influence during prepubertal and the puberty.

The concentration of Cu and Zn showed similar wave pattern. Copper serum concentrations showed an increase at 18 months followed by a decrease then another wave at 21 months which was maintained until the last month of evaluation. Whereas plasma concentration of Zn raised early than Cu, at 17 months of age, followed by a decrease and two others increase at 19 and 22 months of age. Deficiency of Cu and Zn were related with productive, reproductive losses, imune response and body development (HILL; SHANNON, 2019). Meanwhile, studies evaluating serum and plasmatic concentrations of Cu and Zn microminerals are controversial. In our study, the maximum Cu and Zn serum concentrations were 0.56 mg l^{-1} and 0.44 mg l^{-1}, respectively. Mattioli *et al*. (2018) showed different results for plasma concentration of Cu (0.15 mg l^{-1}) and and Zn (1.45 mg l^{-1}) in study with pre-weaning beef calves with 3 months of age, as well as showed by Ramos *et al*. (2013) (2013) in Nellore Bull between 5 and 11 years old, Zn plasma concentrations was 0,006 mg l^{-1}. Another study in Holstein cows (SPO*LDERS* et al., 2010) described that the plasma concentrations of Cu and Zn can reach values of 12.3 µmol l^{-1} and 14.2 µmol l^{-1}, respectively. In these studies, different methods were used to determinated the plasma concentrations for Zn and Cu, with diffent physiological conditions, consequently, is difficult to define Nellore polled bulls with normal or difficiency values. Despite the thresholds for risk of deficiency based on plasma Cu and Zn concentrations remain controversial, the results of the development traits analyzed in our study were in accordance with previous studies and, in our opinion, the concentrations of Cu and Zn did not impair the development of Nellore polled bulls.

CONCLUSION

In conclusion, the parameters analyzed in prepubertal, and pubertal Nellore polled bulls were similar to Bos indicus bulls in the field conditions. The result of this study provides new knowledge in the field of bovine reproduction of Nellore Pulled

Bulls however the possible association between these parameters and the influence of Zn and Cu in puberty remain unclear.

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