

Gonadic Sperm Reserve in Buffaloes Created under Tropical Conditions

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ABSTRACT: The objective of the present study was quantifying the spermatogenic production per gram and daily rate of the testicular parenchyma. Testis of 12 crossbred Murrah buffaloes aged 24 to 48 months created under extensive conditions in the State of São Paulo/Brazil were analyzed. Animals were divided into groups based on the testicular shape (long and long-moderate) and testicular sides (right and left) and the studied parameters were: length and testicular width, weight and volume of the albuginea and mediastinum and net weight of the testicular parenchyma, gonadic sperm reserves, spermatogenic production/g of testicular parenchyma, daily spermatogenic production/g, total daily spermatogenic production, resulting into the values: 8.16 ± 0.87 and 4.29 ± 0.50 cm; 9.09 ± 1.91 g and 8.77 ± 1.88 mL; 0.97 ± 0.39 g and 0.90 ± 0.38 mL; 112.91 ± 18.85 g; $14.32 \times 10^9 \pm 0.15$; $13.42 \times 10^6 \pm 0.17$; $27.40 \times 10^6 \pm 0.35$ and $2.92 \times 10^9 \pm 0.30$, registering no difference between right and left testis in relation to the parameters ($P > 0.05$). There was no relation between testicular biometry and spermatogenic production ($P > 0.05$). According to the obtained values, all animals were considered sexually mature and presented an efficient spermatogenic production per gram of testicular parenchyma and total daily production.

Key words: Buffaloes, Spermatogenic production, Gonadic sperm reserve.

INTRODUCTION - Accumulated growth of the buffalo herd between 1961 and 2005 was about surprising 1.806% (Bernardes, 2006), in Brazil. Buffaloes are animals easily adapted to the Brazilian environment and it is characterized by the good reproductive efficiency and fast ponderal development (Baruselli and Carvalho, 2002). Therefore, the knowledge of the reproductive aspects is a basic requirement for the improvement and evaluation of the fertility of these animals (Oba, 2003). The gonadic sperm reserve is perhaps one of the most important characteristics among the male reproductive characteristics, because it reflects the breeding potential with success that the same can accomplish during the breeding season, being able to establish the male/female relationship without having a decrease of the reproductive efficiency at the end of the breeding season (Guimarães, 1997). The objective of this study was to estimate and evaluate daily and total spermatogenic production in crossbred Murrah buffaloes under tropical conditions.

MATERIAL AND METHODS - There were used 12 crossbred Murrah buffaloes aged 24 to 48 months, abated in the slaughterhouse, located in the municipal district of Lençóis Paulistas/SP. After the slaughter of the animals, the testis were identified and transported under refrigeration to the Department of Animal Reproduction and Veterinary Radiology-FMVZ/UNESP (Botucatu/SP), where the *post-mortem* testicular biometry was accomplished. After that, the testis were stored at -18°C until the day of the processing. The volume and weight of the testis were measured for later dissection of them. The weight and volume of the albuginea and mediastinum were measured by the Scherle (1970) method and the net weight of the testicular parenchyma was determined by subtracting the weight of the albuginea and mediastinum.

The gonadic sperm reserve and the spermatid production/g of testicular parenchyma had been calculated in accordance to the methodology by Amann and Almquist (1962) and adopted by Cardoso (1981). The spermatid daily production/g of testis and total daily spermatid production per testis were calculated using a time divisor of 4.9 days (Sharma and Gupta, 1980), considering that the resistant cells to the homogenizing process are the elongate spermatids, that are between the 4th to 8th stadium of the seminiferous epithelium cycle (Amann et al. 1974).

Descriptive analysis (mean, standard deviation and variation coefficient) had been used for all the studied parameters and variance analysis to evaluate the effect of the right and left testis and of the testicular shape (long and long-moderate) on the studied parameters.

RESULTS AND CONCLUSIONS - The values obtained for testicular biometry, weight and volume of the testicular parenchyma, albuginea and mediastinum are described in table 1. There was no difference observed for right and left testis and for testicular shape ($P>0.05$) in relation to the studied characteristics, except for the testicular width that was larger for the long-moderate testis ($P<0.05$). Similar results were registered by Melo (1991), where the values for weight, length and testicular width didn't differ between right and left testis, in animals from 10 to 24 months of age created extensively. However, Sekharam and Rao (1986) verified smaller length and width values for the left testis in animals even 12 months of age (8.13 x 6.21 vs 8.5 x 6.52 cm).

Ahmad et al. (1989) had registered values of $188\pm12\text{ cm}^3$ for testicular volume in puberal Nili-Ravi buffaloes. Sharma and Gupta (1980) had registered values of 138.6 and 8.45g for testicular parenchyma and albuginea, respectively, in adult buffaloes in sexual rest.

The values obtained for the spermatid production parameters in accordance to side and testicular shape are summarized in table 2. There was observed no difference between right and left testis and between long and long moderate testicular shape for these characteristics ($P>0.05$).

According to McCool and Entwistle (1989a), the spermatid production/g of testicular parenchyma in 146 sexually mature Australian buffaloes was stabilized starting from 3 to 3.5 years of age, with spermatid daily production/g of $14.04\times10^6\pm0.39$ and spermatid daily production of $1.86\times10^9\pm0.07$. The authors observed that the maximum sperm production happened in the end of the rainy season and beginning of the dry season.

According to McCool and Entwistle (1989b), the puberty is reached out when the spermatid production/g of testicular parenchyma is equal or higher than 0.5×10^6 and the sexual maturity with equal or superior values to 14×10^6 .

Table 1. Mean and standard deviation ($M \pm S.D.$) of testicular biometry, weight and volume of the testicular parenchyma, albuginea and mediastinum in buffaloes.

	COMPT	LARGT	VTEST	PEST	PESALB	VOLALB	PESMED	VOLMED
RT	8.07 $\pm 0.92^a$	4.25 $\pm 0.58^a$	108.31 $\pm 22.3^a$	124.12 $\pm 19.5^a$	8.82 $\pm 1.87^a$	8.49 $\pm 1.69^a$	0.95 $\pm 0.33^a$	0.87 $\pm 0.30^a$
LT	8.27 $\pm 0.85^a$	4.34 $\pm 0.39^a$	94.28 $\pm 47.24^a$	125.32 $\pm 20.36^a$	9.41 $\pm 2.02^a$	9.09 $\pm 2.13^a$	0.10 $\pm 0.48^a$	0.92 $\pm 0.46^a$
LG	8.58 $\pm 0.96^a$	3.99 $\pm 4.49^b$	108.29 $\pm 22.18^a$	118.59 $\pm 12.31^a$	9.15 $\pm 1.71^a$	8.78 $\pm 1.72^a$	0.99 $\pm 0.42^a$	0.90 $\pm 0.36^a$
LM	7.87 $\pm 0.70^a$	4.50 $\pm 0.40^a$	112.04 $\pm 20.07^a$	128.89 $\pm 22.64^a$	9.04 $\pm 2.11^a$	8.75 $\pm 2.06^a$	0.96 $\pm 0.40^a$	0.90 $\pm 0.40^a$
$M \pm S.D.$	8.16 ± 0.87	4.29 ± 0.50	110.51 ± 20.49	124.66 ± 19.42	9.09 ± 1.91	8.77 ± 1.88	0.97 ± 0.39	0.90 ± 0.38

RT: right testis; LT: left testis; M: mean and standard deviation; LG: long testicular shape; LM: long moderate testicular shape; COMPT: testicular length in centimeters; LARGT: testicular width in centimeters; VTEST: calculated testicular volume; PEST: testicular weight in grams; PESALB: albuginea weight in grams; VOLALB: albuginea volume in mL; PESMED: mediastinum weight in grams; VOLMED: mediastinum volume in mL.

^{a,b} = Values followed by similar letters in the same column do not differ between itself ($P > 0.05$) by Tukey Test.

Table 2. Mean and standard deviation ($M \pm S.D.$) of the spermatic production in buffaloes.

	PTL	RESGON (10^9)	PROGRAM (10^6)	PRODIAGR (10^6)	PRODIATO (10^9)
RT	113.24 $\pm$ 17.82	11.03 $\pm$ 2.84	97.35 $\pm$ 1.96	19.86 $\pm$ 4.00	2.25 $\pm$ 0.005
LT	112.53 $\pm$ 21.00	11.14 $\pm$ 2.16	178.54 $\pm$ 2.51	36.44 $\pm$ 5.12	3.73 $\pm$ 0.004
LG	107.20 $\pm$ 14.72	10.17 $\pm$ 3.17	94.24 $\pm$ 2.52	19.23 $\pm$ 5.10	2.07 $\pm$ 0.006
LM	116.87 $\pm$ 20.89	17.20 $\pm$ 1.88	16.19 $\pm$ 2.19	33.05 $\pm$ 4.48	3.51 $\pm$ 0.003
$M \pm S.D.$	112.91 $\pm$ 18.85	14.32 $\pm$ 0.001	13.42 $\pm$ 1.70	27.40 $\pm$ 3.47	2.92 $\pm$ 0.003

RT: right testis; LT: left testis; M: mean and standard deviation; LG: long testicular shape; LM: long moderate testicular shape; PTL: testicular net weight testicular in grams; RESGON: gonadic sperm reserves; PROGRAM: spermatic production/g of testicular parenchyma; PRODIAGR: daily spermatic production/g; PRODIATO: total daily spermatic production.

Similar values were registered by Sharma and Gupta (1980), with the efficiency of the spermatic production/g of testicular parenchyma and total daily spermatic production of 2.02×10^9 spermatozoons per testis, being inferior to the registered in the present study.

There was no relationship between the testicular biometry and the spermatic production ($P > 0.05$). Although the amplitude of the age band, the biometrical and quantitative data of the testis presented low variation coefficient, indicating homogeneity in the studied group.

In accordance to the obtained data, all the animals were sexually mature and presented efficient spermatid production/g of testicular parenchyma and total daily production.

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REFERENCES - **Ahmad**, N., Shahah, M., Khurshid, S., Arslan, M., 1989. Pubertal development in the male buffalo: Longitudinal analysis of body growth, testicular size and serum profiles of testosterone and oestradiol. *Anim. Reprod. Sci.* 19:161-170. **Amann**, R.P., Almquist, J.O., 1962. Reproductive capacity of dairy bulls. Direct and indirect measurement of testicular sperm production. *J. Dairy Sci.* 45:774. **Amann**, R.P., Kavanaugh, J.F., Griel, L.C. Jr., Voglmayr, J. K., 1974. Sperm production of Holstein bulls determined from testicular spermatid reserves, after cannulation of rete testis or vas deferents, and by daily ejaculation, 1974. *J. Dairy Sci.* 57:93-99. **Bernardes**, O., 2006. Os búfalos no Brasil. In: III Simpósio Búfalos de las Américas. MEMORIAS. 2nd Buffalo Symposium of the Europe and Americas. 14-20. **Baruselli**, P. S., Carvalho, N. A. T., 2002. Reproduction Management and artificial insemination in buffalo. In: 1 Buffalo Symposium of Americas, Pará. 119-143. **Cardoso**, F.M. 1981 Morfologia, cinética e quantificação da espermatogênese em zebus (*Bos indicus*). Belo Horizonte: Instituto de Ciências Biológicas-UFMG. 208 (Tese de Doutorado em Ciências, área de concentração em morfologia). **Guimarães**, J. D. Avaliação andrológica e estudos quantitativos e qualitativos da espermatogênese de touros mestiços F1 Holandes x Zebu e Red Angus x Zebu. Tese (doutorado): 186 **Mccool**, C. J., Entwistle, K. W., 1989a. The development of puberty and sexual maturity in the Australian Swamp buffalo bull. *Theriogenology*. 32:171-184. **Mccool**, C. J., Entwistle, K. W., 1989b. Reproductive function in the Australian Swamp buffalo bull: Age effects and seasonal effects. *Theriogenology*. 31:583-594. **Melo**, M. I. V. 1991. Desenvolvimento testicular e dinâmica da espermatogênese de búfalos mestiços de 10 a 24 meses de idade. Dissertação (Mestrado). Universidade Federal de Minas Gerais, escola de veterinária. Belo Horizonte: 66 **Oba**, 2003 E. Tópicos atualizados ligados à reprodução na espécie bubalina. In: Contribuição ao estudo dos bubalinos: período de 1972-2001:palestras. Botucatu, 578p. **Scherle**, W. A., 1970. simple method for volumetry of organs in quantitative stereology. *Mikroskopie*, 26:57-60. **Sekharam**, G. S., Rao, A. R., 1986. Biometry of testes and semen characteristics of Murrah buffalo bulls. *Indian Veterinary Journal*. 63:228-232. **Sharma**, A. K., Gupta, R. C., 1980. Duration of seminiferous epithelial cycle in buffalo bulls (*Bubalus bubalis*). *Animal Reproduction Science*. 3:217-224.