

Seasonal Effects on Semen and Testosterone in Zebu and Taurine Bulls

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ABSTRACT

Background: In Brazil, a higher percentage of abnormal sperm after high temperature and relative humidity observed a higher semen volume in the rainy. The semen quality may be significantly affected by environment, by fluctuations in temperature, humidity and photoperiod. The objective of this study was to evaluate the seasons influence (spring, summer, autumn and winter) on semen characteristics and testosterone levels in Nellore and Simmental bulls raised on field.

Materials, Methods & Results: The city of Presidente Prudente-SP-Brazil, where the experiment was realized, presents latitude 21°29'50"S, longitude 49°14'2"W and altitude of 475 meters. In 2008/2009, the climatic factors measured for Spring - SP; Summer - SU; Autumn - A and the Winter - W were: average temperature (SP - 25.9; SU - 26.6; A - 21.9 and W - 21.1°C), stroke (SP - 400.9; SU - 464.0; A - 721.3 and W - 142.6 hours), cumulative rainfall (SP - 291.8; SU - 925.0; A - 273.0; and W - 191.8 mm) and average relative humidity (SP - 65.9; SU - 71.7; A - 70.1 and W - 66.7%). Five Nellore and five Simmental bulls with 48-72 months old, extensively managed were evaluated for sexual soundness using physical and morphological characteristics of semen and testosterone serum levels. Sixteen semen samples by automatic electroejaculation were carried out in each of 10 bulls, totaling 160 samples. The experiment was conducted from September to August and made four harvests in each of the four seasons. The analysis of testosterone concentration (ng/dL) were performed with commercial kits and quantification of the hormone made by radioimmunoassay (RIA) totalizing eight replicates per bull, 2 per season, totalizing 80 samples. Data on characteristics of semen and testosterone evaluation were subjected to analysis of variance, considering the study as a factorial experiment, and the differences between means compared by Tukey test at 5%. There was decreased motility and vigor semen ($P < 0.05$) during winter in Simmental bulls. There was correlation between serum testosterone ($P < 0.01$) to motility and vigor in Nellore and Simmental bulls. In relation to sperm morphology, major and total defects were different ($P < 0.05$) between breeds in the spring, with higher percentages observed in the Simmental breed. In the winter, for minor and total defects, there were differences between breeds ($P < 0.05$) being major to the Simmental. For the Simmental breed, there was difference ($P < 0.05$) between summer and winter seasons for the major sperm defects and total quality with better morphology in the summer. The sperm concentration, between breeds, in spring and autumn showed differences ($P < 0.05$), being higher for the Simmental. There was difference ($P < 0.05$) between breeds for testosterone, in the summer season. In the Simmental breed there was a significant correlation between testosterone and semen characteristics, being mass movement (0.31), concentration (0.37) and total defects (-0.26). For the major and total defects in the Simmental, average correlations were obtained in relation to testosterone.

Discussion: The seasonal variation has been studied with a focus on physical characteristics and sperm morphology in cattle in the tropics, describing the decline in semen quality can occur due to thermal discomfort of the animals. Based on the data it is possible to conclude that the results showed a hormonal and semen characteristics variation throughout the season change the semen quality. Bulls from Nellore breed differed from Simmental, demonstrating adaptability to the field conditions.

Keywords: bovine, sazonalidade, espermatozoa, endocrinologia, RIA.

INTRODUCTION

The seasonal variation has been studied with a focus on physical characteristics and sperm morphology in cattle in the tropics, describing the decline in semen quality can occur due to thermal discomfort of the animals on high temperatures [10]. The decrease in progressive motility, the reduction of sperm production and increase the percentage of spermatozoa with morphological changes are caused by a moderate elevation of testicular temperature [13].

In Brazil, a higher percentage of abnormal sperm after high temperature and relative humidity observed a higher semen volume in the rainy [15]. Studying the Nellore, concluded that semen quality may be significantly affected by environment, by fluctuations in temperature, humidity and photoperiod [9]. Studies on the environmental influence on physical characteristics and sperm morphology in bulls have been conducted in artificial insemination centers, with animals in permanent regime of semen collection [3]. Was found a positive correlation between serum testosterone concentration and fertility of bulls, it was also found that animals with higher sperm motility showed, on average, higher concentration of testosterone [1]. Considering the predominance of extensive farming and natural mating in Brazil becomes important to investigate the seasons influence on semen characteristics of bulls raised on field. The objective of this study was to evaluate the seasons influence (spring, summer, autumn and winter) on semen characteristics and testosterone levels in Nellore and Simmental bulls raised on field.

MATERIALS AND METHODS

This experiment was approved by the Ethical Committee - University of Oeste Paulista - UNOESTE, Presidente Prudente - SP, Brazil. Have been utilized bulls of the Nellore (n = 5) and Simmental (n = 5), aged between 48 and 72 months, raised extensively on pasture of *Brachiaria decumbens* with mineral and water *ad libitum*. The city of Presidente Prudente-SP-Brazil, where the experiment was realize, presents latitude 21°29'50"S, longitude 49°14'2"W and altitude of 475 meters. In 2008/2009, the climatic factors measured for Spring - SP; Summer - SU; Autumn - A and the Winter - W were: average temperature (SP - 25.9; SU - 26.6; A - 21.9 and W - 21.1°C), stroke (SP - 400.9; SU - 464.0; A - 721.3 and W - 142.6 h), cumulative rainfall (SP - 291.8; SU - 925.0; A - 273.0; and W - 191.8 mm) and average relative humidity (SP - 65.9; SU - 71.7;

A - 70.1 and W - 66.7%). The climate is characterized by the presence of air masses and tropical polar winter season with a cool and dry and hot summer and rainy.

Sixteen semen samples by electroejaculation¹ automatic were carried out in each of 10 bulls, totaling 160 samples. The experiment was conducted from September to August and made four harvests in each of the four seasons. The morphological sperm were classified according to Barth and Oko [2]. At the same handling of semen collection blood samples were collected by jugular venopunction, and the material centrifuged² at 1500 g / 15 min, the serum separated and stored in cryotubes of 1.5 mL; identified and stored in freezer³ at -20°C until processing. The analysis of testosterone concentration (ng/dL) were performed with commercial kits⁴, solid phase, and quantification of the hormones made by radioimmunoassay (RIA) totalizing eight replicates per bull, 2 per season, totalizing 80 samples. Data on characteristics of semen and hormone were subjected to analysis of variance, considering the study as a factorial experiment, and the differences between means compared by Tukey test at 5%.

RESULTS

For the volume of ejaculate in the Simmental breed, there was difference ($P < 0.05$) between seasons, being higher in summer than winter (Table 1). In summer and autumn, Nellore has lower volume compared to the Simmental ($P < 0.05$).

In winter, the motility and vigor decreased in the Simmental breed, when compared to spring and summer ($P < 0.05$) (Table 1). In relation to sperm morphology, major and total defects were different ($P < 0.05$) between breeds in the spring, with higher percentages observed in the Simmental breed. In the winter, for minor and total defects, there were differences between breeds ($P < 0.05$) being major to the Simmental. For the Simmental breed, there was difference ($P < 0.05$) between summer and winter seasons for the major sperm defects and total quality with better morphology in the summer. The sperm concentration, between breeds, in spring and autumn showed differences ($P < 0.05$), being higher for the Simmental.

There was difference ($P < 0.05$) between breeds for testosterone, in the summer season (Table 2). In the Simmental breed there was a significant correlation between testosterone and semen characteristics, being mass movement (0.31), concentration (0.37) and total defects (-0.26). For the major and total defects in the Simmental, average correlations were obtained in relation to testosterone.

Table 1. Means and standard deviations for semen characteristics of Simmental (S) and Nellore (N) bulls raised extensively in four seasons.

Variable	breed	spring	summer	autumn	winter
vol	S	8.80 ± 0.65Aab	9.85 ± 0.65Aa	8.76 ± 0.58Aab	7.35 ± 0.65Ab
	N	7.10 ± 0.65Aa	7.55 ± 0.65Ba	6.26 ± 0.58Ba	6.05 ± 0.65Aa
mot	S	70.00 ± 5.83Aa	70.00 ± 5.83Aa	60.80 ± 8.21Aab	48.00 ± 5.83Ab
	N	63.50 ± 5.83Aa	60.00 ± 5.83Aa	54.40 ± 5.21Aa	53.50 ± 5.83Aa
vig	S	3.35 ± 0.28Aa	3.55 ± 0.28Aa	3.00 ± 0.25Aab	2.20 ± 0.28Ab
	N	3.05 ± 0.28Aa	2.95 ± 0.28Aa	2.32 ± 0.25Aa	2.50 ± 0.28Aa
Mdef	S	11.33 ± 1.31Aab	8.00 ± 1.07Ab	10.75 ± 0.98Aab	12.18 ± 1.20Aa
	N	6.30 ± 1.07Ba	6.31 ± 1.10Aa	9.92 ± 0.96Aa	9.42 ± 1.10Aa
mdef	S	7.88 ± 0.83Aa	7.25 ± 0.79Aa	8.25 ± 0.72Aa	10.25 ± 0.88Aa
	N	7.60 ± 0.79Aa	5.63 ± 0.81Aa	7.16 ± 0.70Aa	6.84 ± 0.81Ba
Tdef	S	19.22 ± 1.63Aab	15.25 ± 1.54Ab	19.00 ± 1.41Aab	22.43 ± 1.73Aa
	N	13.90 ± 1.53Ba	11.97 ± 1.59Aa	17.08 ± 1.38Aa	16.36 ± 1.59Ba
conc	S	1.35 ± 0.13Aa	1.38 ± 0.13Aa	1.32 ± 0.12Aa	1.00 ± 0.13Aa
	N	0.88 ± 0.13Ba	1.14 ± 0.13Aa	0.95 ± 0.12Ba	0.91 ± 0.13Aa

^{a,b}Different letters in line indicate significant differences ($P < 0.05$); ^{A,B}Different letters in column ($P < 0.05$), volume-mL (vol), motility-% (mot), sperm vigor-1 to 5 (vig), sperm concentration-x10⁹/mL (conc), major defects-% (Mdef), minor defects-% (mdef), total defects-% (Tdef).

Table 2. Means and standard deviations for serum testosterone (ng/dL) of Simmental (S) and Nellore (N) bulls raised extensively in four seasons.

Variable	breed	spring	summer	autumn	winter
Testosterone	S	879.4 ± 164.7Aa	901.0 ± 173.6Aa	584.0 ± 164.7Aa	648.1 ± 164.7Aa
	N	430.4 ± 173.6Aa	234.7 ± 196.8Ba	420.3 ± 164.7Aa	329.1 ± 164.7Aa

^aSame letters in line ($P > 0.05$); ^{A,B}Different letters in column ($P < 0.05$).

Table 3. Correlations between testosterone (ng/dL) and semen characteristics of Simmental (S) and Nellore (N) breeds raised extensively in four seasons.

breed	season	Test	Average									
			Semen									
			vol	col	asp	mm	mot	vig	conc	Mdef	mdef	Tdef
S	sp	879.5	8.8	1.7	1.8	1.8	67.0	3.0	1.09	13.8	7.8	21.7
	s	901.1	9.4	2.1	1.8	4.0	80.0	4.1	1.69	6.2	6.2	12.4
	a	584.0	10.8	1.4	1.4	2.8	64.0	3.2	1.36	10.8	7.4	18.2
	w	648.2	7.5	1.2	1.9	1.8	46.0	2.1	0.96	10.8	8.2	19.1
	correlations			-0.16	0.80	0.62	0.31	0.69	0.57	0.37	-0.19	-0.47
N	sp	430.4	8.2	1.4	1.8	2.2	63.3	3.1	0.91	5.8	7.0	12.8
	s	234.7	8.2	3.0	2.4	3.7	80.0	4.0	1.32	5.8	4.5	10.4
	a	420.3	5.7	1.6	1.5	1.3	33.0	1.5	0.70	10.7	8.0	18.7
	w	329.1	5.8	2.2	2.0	2.2	56.0	2.6	1.08	9.2	7.5	16.9
	correlations			-0.28	-1.00	-0.89	-0.87	-0.71	-0.70	-0.93	0.36	0.82

spring (sp), summer (s), autumn (a) and winter (w). Volume-mL (vol), color (col): 1-milky, 2-milky white and 3-white marble, aspect (asp): 1-aquous, 2-viscous and 3-creamy; mass movement (mm)-1 to 5, sperm motility-% (mot), sperm vigor-1 to 5 (vig), sperm concentration-x10⁹/mL (conc), major defects-% (Mdef), minor defects-% (mdef), total defects-% (Tdef).

DISCUSSION

The larger volume of ejaculate in summer may be related to the rainfall at the station 925 mm versus 191.8 mm in winter, resulting in a higher percentage of water in the grass, according to [15]. For the volume of ejaculate there is variation among authors. Related in Pakistan range from 3.84 ± 0.06 mL and 4.15 ± 0.05 mL for Holsteins and 2.79 ± 0.03 mL and 3.02 ± 0.03 mL for the Jersey breed during year with a drop of semen quality in warmer weather station [8]. In research conducted in Romania with Simmental bulls gained between 5.98 mL and 11.59 mL during the year, describing that temperature above 20°C are detrimental to sperm quality [11]. It is noteworthy that the method of collection by electroejaculation can influence the volume of semen obtained, in the present study this factor is minimized by using standardized automatic device with pulses during the harvest.

In Brazil, obtained increase of sperm motility in Nellore in the rainy season (summer) [14]. However, in the present study similar sperm motility in same breed was observed among seasons. In winter, for Nellore, values for motility and vigor were similar to those obtained in Mato Grosso do Sul, Brazil in the same season [7], probably due to the geographical proximity of the experiments. For the Simmental breed, the results were similar checking drop in motility and vigor in the dry season (winter) [14]. In relation to sperm morphology it is noteworthy that despite the differences between breeds for this variable, one should consider the peculiarities and specificities of the different genetic backgrounds compared. In the present study possibly the better sperm morphology for the Simmental breed in the summer has relation with the best nutritional support that may have contributed positively in spermatogenesis, a process dependent of testosterone synthesis.

In Nellore bulls, obtained a lower percentage of major defects in the rainy season (warm) [14]. However, the sperm morphology values from our study were not different between seasons. The sperm concentration in spring and autumn was higher for the Simmental. This result expected due to the larger size of the genital organs in this breed. The concentration for testosterone in Nellore bulls were similar in same breed with average levels of testosterone in the winter and spring of 124 ng/dL [4]. The levels also were similar to [6] survey conducted in October-December

(spring), reporting differences ($P < 0.05$) between the Brown-Swiss breed (116.60 ± 39.03 ng/dL) and Brangus (230.92 ± 27.60 ng/dL) for testosterone levels.

For the Nellore bulls, a decrease in testosterone levels in summer, possibly due to the freedom of animals to physical activity, associated with a higher average temperature (26.8°C). In winter season, with average temperature lower (21.1°C), associated with decreased quality of grass, testosterone levels were lower similar to [4]. The level of individual adaptation to climate can influence testosterone levels, being studied by this consideration in bulls [5], reporting different levels of testosterone among treatments with 24.04 ± 5.89 ng/dL for the thermoneutral group and 49.85 ± 6.83 ng/dL for the group subjected to heat stress. The results of this study were similar to [12] who performed measurements of testosterone levels in cattle, describing the winter average of 50 ng/dL, in the fall 70 ng/dL, and in the spring and summer of 130 ng/dL. In the Simmental breed there was a significant correlation between testosterone and semen characteristics. These results are according [1] with a significative correlation between concentration of serum testosterone and semen quality in bulls.

For the major and total defects in the Simmental, correlations were obtained in relation to testosterone. It is worth mentioning that the presence of sperm defects minor, major and total, regardless of the season for the two breeds, did not reveal percentages exceeding the acceptable levels for reproductive activity.

There is a need for further research to better understand the influence of seasons on the reproduction of bulls bred extensively in Brazilian conditions, allowing a better exploitation of these players at particular times of year.

CONCLUSION

Based on our results it is possible conclude that the Nellore and Simmental bulls produced good quality of semen over the seasons. For the Simmental breed, the seasons of spring and summer affected the improvement in winter and reduced sperm motility and vigor. The winter compared to summer on a larger scale affected the morphology. The quality of semen showed high correlation with testosterone. For Nellore no significant variation was observed in the characteristics of semen over the seasons.

SOURCES AND MANUFACTURERS

¹NEOVET, Autoejac®, Uberaba, Brazil.

²FANEM, Baby 2 - 206R®, São Paulo, Brazil.

³ELECTROLUX, FFE 24®, São Paulo, Brazil.

⁴DPC-Medlab®, São Paulo, Brazil.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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