Breed, age, and environmental effects on bull semen in the low tropics

Efectos de raza, edad y medio ambiente en el semen de toros del trópico bajo

Efeitos da raça, idade e ambiente no sêmen de touros dos baixos trópicos

Jesús Alfonso Sánchez Viafara[1, 2]* Ph.D
Greysy Dariana Vergel Martínez[1] MVZ
Breysy Yuliana Vergel Martínez[1] MVZ
Julian Valencia[3] Ph.D.

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1 Universidad de Santander, Facultad de Ciencias Agrícolas y Veterinarias, Valledupar, Colombia.

2 Centro de Desarrollo Tecnológico del Cesar, CDT Cesar. Unidad de Investigación Ganadera, Grupo Investigación y Desarrollo en Sistemas Agropecuarios, Valledupar, Cesar, Colombia.

3 Antonio Nariño University, Faculty of Veterinary Medicine, Popayán, Colombia.

* Correspondence: jesusveterinario85@hotmail.com

Abstract

Objective. This study aimed to identify factors that affected the semen quality of breeding bulls in the low tropics of Colombia.

Materials and methods. The rest period, breed (B), and environmental (E) effects on sperm concentration and semen volume were examined in a first analysis with 399 bulls. Subsequently, breed and environmental effects on sperm concentration, semen volume, and progressive motility, in addition to breed (breed or crossbreed) effects on scrotal circumference, were examined in a second analysis performed with 1848 bulls.

Results. The scrotal circumference of Jersey×Gyr bulls was significantly (p<0.05) higher than that of different breeds and F1 bulls. In addition, significant differences were also found in semen variables (p<0.01), including sperm concentration (p<0.05), which varied with the environmental conditions and was significantly higher in altitudinal zone 4 (AZ4: 150–199 meters above sea level (MASL) altitude, 33.9°C environmental temperature (ET), and 71.2% relative humidity (RH)) and was significantly lower in AZ5 (altitude higher than 500 MASL, 23°C ET, and 76% RH). In turn, the following correlations were found: the higher the semen volume, the higher progressive is the motility and sperm concentration, and the higher the sperm concentration, the higher is the progressive motility.

Conclusions. AZ5 affected sperm concentration, but no significant differences were found in sperm concentration or semen volume between the breeds under study.

Keywords: animal reproduction, bulls, testicles, semen *(Sources: thesaurus biología animal)*

Resumen

Objetivo. Este estudio tuvo como objetivo identificar los factores que afectaron la calidad seminal de toros reproductores en el trópico bajo de Colombia.

Materiales y métodos. Los efectos del período de descanso, la raza (B) y el medio ambiente (E) sobre la concentración de esperma y el volumen de semen se examinaron en un primer análisis con 399 toros. Posteriormente, los efectos de la raza y el medio ambiente sobre la concentración de esperma, el volumen del semen y la motilidad progresiva, además de los efectos de la raza (raza o cruzamiento) sobre la circunferencia escrotal, se examinaron en un segundo análisis realizado con 1848 toros.

Resultados. La circunferencia escrotal de los toros Jersey×Gyr fue significativamente (p<0.05) mayor que la de las diferentes razas y los toros F1. Además, también se encontraron diferencias significativas en las variables seminales (p<0.01), incluida la concentración de espermatozoides (p<0.05), que varió con las condiciones ambientales y fue significativamente mayor en la zona altitudinal 4 (AZ4: 150-199 MSNM). 33,9°C de temperatura ambiental (ET) y 71,2% de humedad relativa (HR)) y fue significativamente menor en AZ5 (altitud superior a 500 msnm, 23°C ET y 76% HR). A su vez, se encontraron las siguientes correlaciones: a mayor volumen seminal, mayor progresiva es la motilidad y concentración espermática, y a mayor concentración espermática, mayor es la motilidad progresiva.

Conclusiones. AZ5 afectó la concentración de espermatozoides, pero no se encontraron diferencias significativas en la concentración de espermatozoides o el volumen de semen entre las razas en estudio.

Palabras clave: reproducción animal, toros, testículos, semen *(Sources: thesaurus biología animal)*

Resumo

Mirar. Este estudo teve como objetivo identificar os fatores que afetaram a qualidade do sêmen de touros reprodutores nos trópicos baixos da Colômbia.

Materiais e métodos. Os efeitos do período de descanso, raça (B) e ambiente (E) na concentração espermática e no volume de sêmen foram examinados em uma primeira análise com 399 touros. Posteriormente, os efeitos da raça e do ambiente na concentração espermática, no volume do sêmen e na motilidade progressiva, bem como os efeitos da raça (raça ou cruzamento) na circunferência escrotal, foram examinados em uma segunda análise realizada com 1.848 touros.

Resultados. A circunferência escrotal dos touros Jersey×Gyr foi significativamente (p<0,05) maior que a das diferentes raças e dos touros F1. Além disso, também foram encontradas diferenças significativas nas variáveis seminais (p<0,01), incluindo a concentração espermática (p<0,05), que variou com as condições ambientais e foi significativamente maior na zona altitudinal 4 (AZ4: 150-199 msnm). 33,9°C de temperatura ambiente (ET) e 71,2% de umidade relativa (h)) e foi significativamente menor na AZ5 (altitude superior a 500 metros acima do nível do mar, 23°C ET e 76% UR). Por sua vez, foram encontradas as seguintes correlações: quanto maior o volume do sêmen, maior a motilidade progressiva e a concentração espermática, e quanto maior a concentração espermática, maior a motilidade progressiva.

Conclusões. O AZ5 afetou a concentração espermática, mas não foram encontradas diferenças significativas na concentração espermática ou no volume de sêmen entre as raças estudadas.

Palavras-chave: reprodução animal, touros, testículos, sêmen (Fontes: Thesaurus Animal Biology)

Introduction

Bull fertility directly affects the reproductive rates of farms. Although andrological studies have been underestimated in general, the use of breeders without a reproductive evaluation leads to large economic losses, decreasing livestock productivity. The main causes of bull infertility are associated with inadequate health and nutrition management [1].

Considering the importance of bulls for livestock farming, each bull must undergo an andrological examination to establish its reproductive potential and to ensure the fertility and production rates of the system towards reducing the calving-to-conception interval (days open) of cows and cutting maintenance costs of infertile animals [2]. However, farmers lack knowledge about the reproductive parameters that should be analyzed when selecting a breeder for their farm. Farmers tend to select breeders primarily for their phenotypic traits, which are less functional [3].

The andrological evaluation of a bull is a simple, practical, and inexpensive for identifying animals with reproductive problems whose semen alterations can remain undetectable to the naked eye and affect the production of a livestock farm [2]. Various factors affect both the onset and outcome of reproductive events in bulls, such as breed, environment, and nutrition and health management, in both beef and dairy cattle [4].

The most important reproductive parameters include those that reflect the onset of puberty and sexual activity, which express fertility at different stages of the reproductive life. However, proper animal management is necessary to increase productivity and therefore profitability [4].

Considering the above, some parameters of the sperm quality of breeding bulls in different municipalities of the department of Cesar, Colombia were evaluated through a retrospective study, thereby assessing factors that affect sperm quality, including breed, age, and environmental conditions. These results enable small and medium farmers to identify the parameters that must be selected to avoid maintaining animals that negatively affect the reproductive performance of the herd.

Material and methods

Study type. In this study, no animal experiments were performed, only retrospective analyzes of the parameters observed in andrological evaluations made in the Cesar state.

Study location. The study was conducted in 23 municipalities of the Cesar department, which is located in northeastern Colombia (07º41’16’’ and 10º52’14’’ north latitude and 72º53’27’’ and 74º08’28’’ west longitude), covers an area of 22,905 km², and has a distinctly tropical climate; however, given the elevation of large land sectors, this region presents a wide climatic variety with various altitudinal zones where temperatures surpass 28°C, averaging 20°C. Sperm were collected in the year 2020 between the months of March to December.

Study animals. To assess breed and environmental effects on sperm concentration and semen volume, in addition to determining the relationship between breed and scrotal circumference, 1848 breeding bulls, 2 to 10 years old, weighing between of 300 kg to 800 kg of the breeds 3/4 SenepolxRomosinuano (n=4), Beefmaster (n=13), BON (n=4), Brahaman (n=312), White Brahman (n=99), Grey Brahman (n=222), Brahman-Gyr (n=27), Brahman-Holstein (n=5), Brahman-Brown Swiss (n=5), Red Brahman (n=301), Red Brahman-Gyr (n=6), Bramolando (n=4), Brangus (n=9), Black Brangus (n=21), Red Brangus (n=7), Zebu (n=15), Zebu-Gyr (n=4), Zebu Simmental (n=8), Colombian criollo (n=25), Guzerat (n=67), Guzerat-Gyr (n=4), Gyr (n=257), Gyr-Zebu (n=10), Gyr-Holstein (n=4), Girolando (n=95), Holstein (n=17), Holstein-zebu (n=15), Jersey x Gyr (n=12), Gyr (crossbreed) (n=11), Holstein (crossbreed) (n=14), Simental (crossbreed) (n=18), Brown Swiss (n=28), Brown Swiss-Zebu (n=23), Romosinuano (n=9), Senepol (n=12), Simbrah (n=82), Simental (n=43), Simental-Gyr (n=7) and Brahman (crossbreed) (n=29) were evaluated under different environmental conditions (E) established as previously reported according to the following altitudinal zones: AZ1: 50–99 meters above sea level (MASL) altitude, 32.8°C average environmental temperature (ET), and 79% relative humidity (RH); AZ2: 100-149 MASL altitude, 33.8°C ET, and 79.4% RH; AZ3: 150-199 masl altitude, 33.9°C ET, and 71.2% RH;, AZ4: 200-500 MASL altitude, 34°C ET, and 72% RH; AZ5: altitude higher than 500 MASL, 23°C ET, and 76% RH. The animals were grazed and fed on native forage.

At the corral, the bulls were subjected to a general physical examination, inspecting the legs to identify alterations in the musculoskeletal system and evaluating the body condition (BC). The bulls of beef and dual-purpose breeds were scored on a scale ranging from 1 to 9 (where 1 represents an extremely skinny bull and 9 represents an excessively fat bull). The bulls of dairy breeds were scored on a scale ranging from 1 to 5 (where 1 represents an extremely skinny animal and 5 represents an obese animal).

The animals were taken to the cattle chute, where the external breeding organs, such as foreskin, penis, scrotum, and testicles, were examined by inspection and palpation, examining the size, shape, consistency, thickness, and integrity of the skin to detect possible abnormalities. The scrotal circumference (SC) was then determined by measuring its perimeter using a tape measure.

Internal reproductive organs, such as seminal vesicles, the inguinal ring, and the prostate, were then examined by rectal palpation. Subsequently, the foreskin hairs were cut, and preputial washing was performed to avoid contaminating the sperm sample. Sperm was collected by introducing the rectal ejaculatory probe into the rectum and adapting this electric probe to the electroejaculator to induce electrical impulses in automatic mode.

Once the semen sample was collected, the semen was subjected to a macroscopic evaluation, which included color, volume, appearance, and pH. Lastly, a microscopic examination of the sample was performed, assessing mass motility, individual motility, viability, and sperm concentration.

This information was recorded in an Excel spreadsheet, which included data such as breed, weight, age, tag, name of the owner, name of the veterinarian, AZ, and results of andrological tests for subsequent analysis.

The rest period (<5 days, 5 to 15 days, 16 to 30 days, 31 to 60 days, 61 to 90 days and >90 days), breed (breed or crossbreed), and environmental (ET, RH, and altitude (MASL)) effects on sperm concentration and semen volume were assessed in 399 bulls of different breeds from different farms located in the Cesar department. For this purpose, the data were organized in a spreadsheet with information from andrological evaluations to assign a numerical code to each item.

Data management and statistical analysis. Data were evaluated for normality and homogeneity of variance using the Shapiro-Wilk and Levene tests. In both analyses, a general linear model was performed using Tukey's test for multiple comparisons.

Additionally, correlation analysis was performed between the variables of interest. Data analysis was performed using the statistical software package IBM SPSS for Windows (Version 23.0; Chicago, IL, USA) and the graphs were constructed using the software GraphPad Prism 8 (Version 8.01 GraphPad Software Inc., San Diego, CA, USA). The results are expressed as mean ± standard error of the mean (SEM), and the significance level was set at $p<0.05$.

Results

The first analysis aimed at identifying the breed or crossbreed under study with the greatest scrotal circumference (Figure 1), which was the crossbreed Jersey×Gyr ($p<0.05$).

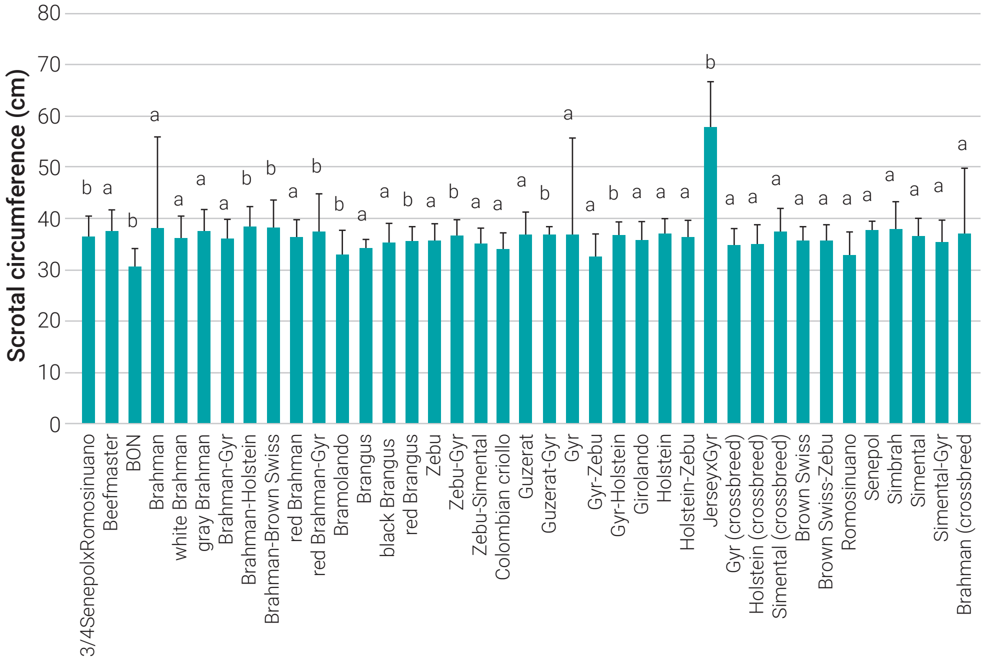

Figure 1. Scrotal circumference by breed and crossbreed

This first analysis showed significant ($p<0.05$) breed (breed or crossbreed) and environmental effects on sperm concentration and semen volume. However, the rest period (for example, interval between ejaculations) effect on the same variables (Figures 2 and 3) was nonsignificant ($p>0.05$). In turn, no significant effect on progressive motility was found in the first analysis (Table 1).

Table 1. Rest period (interval between ejaculations) effect on progressive motility in bulls from different municipalities of the Cesar department

Origin	Type III sum of squares	gl	Root mean square	F	*p*-value
Adjusted model	2138.732a	5	427.746	1.499	0.190
Intersection	944369.110	1	944369.110	3308.840	0.000
Rest	2138.732	5	427.746	1.499	0.190
Error	97324.092	341	285.408		
Total	2041600.000	347			
Adjusted Total	99462.824	346			

Note a. R squared = 0.022 (adjusted R squared = 0.007)

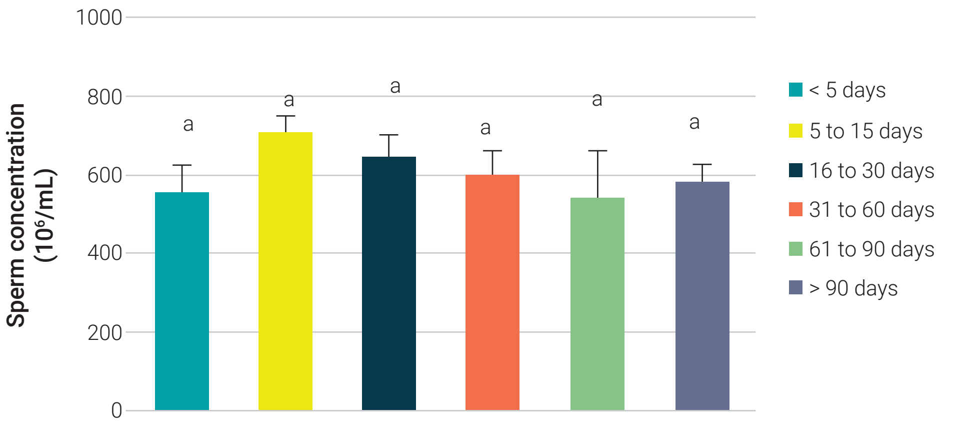

Figure 2. Rest period effect on sperm concentration

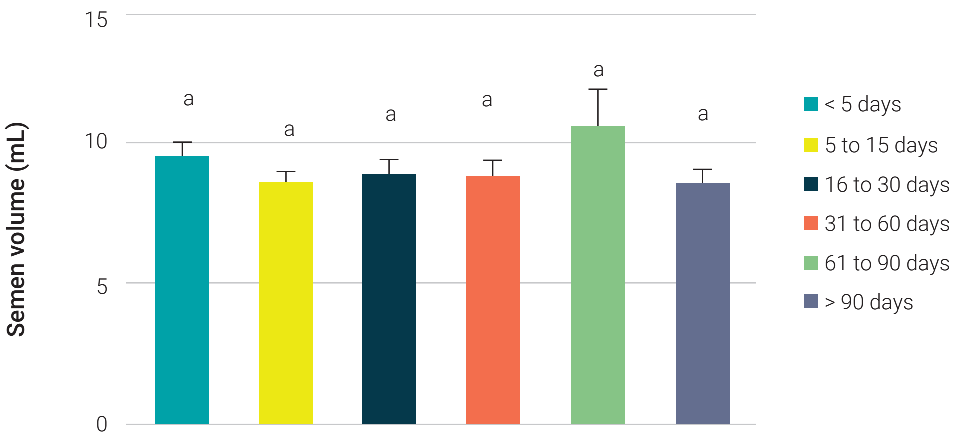

Figure 3. Rest period effect on semen volume

In the second analysis, significant differences were found in sperm concentration as a function of environmental conditions, indicating that sperm concentration was significantly higher ($p<0.05$) in AZ4 (150-199 MASL altitude, 33.9°C ET, and 71.2% RH) and significantly lower in AZ5 (altitude higher than 500 MASL, 23 °C ET, and 76% RH) (Figure 4). The factor AZ significantly ($p>0.05$) affected the variable semen volume in the present study (Figure 5).

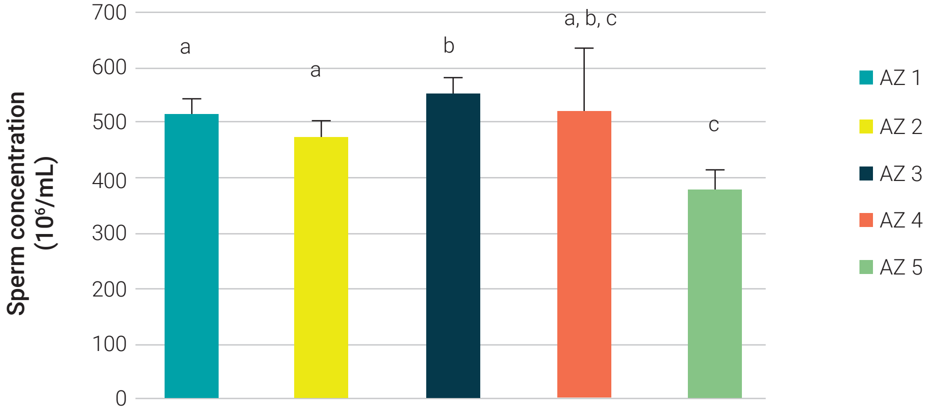

Figure 4. Altitudinal zone (AZ) effect on sperm concentration

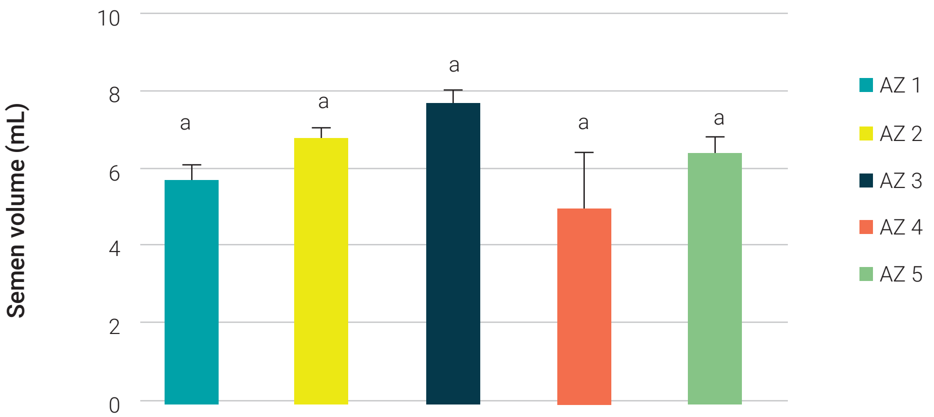

Figure 5. Altitudinal zone effect on semen volume

Similarly, in the second analysis, differences in sperm concentration were assessed between different breeds and crossbreeds (Table 2). The breed Brahman had a higher ($p<0.05$) sperm concentration than White Brahman, Red Brahman, Grey

Brahman, Gyr, Half-blood Simmental, Brown Swiss, and Simbrah. Conversely, the breed White Brahman had a lower sperm concentration than the breeds Brahman, Gyr, and Brown Swiss-Zebu. In turn, the breed Grey Brahman had a lower sperm concentration than the breeds Brahman, Brahman-Gyr, Colombian criollo, Gyr, Girolando, and Brown Swiss-Zebu, whereas the breed Brahman-Gyr had a higher sperm concentration than Brahman gris and Half-blood Simmental. However, Red Brahman had a significantly ($p<0.05$) lower sperm concentration than Brahman, Gyr, and Brown Swiss Zebu. By contrast, Colombian criollo bulls had a significantly ($p<0.05$) higher sperm concentration than Grey Brahman bulls.

Table 2. Comparison of sperm concentration between breeds and crossbreeds

(I) Breed		Difference of means (I-J)	SEM	Sig.
Brahman	White Brahman	230.1658*	32.0544901	9.68916E-10
	Grey Brahman	289.0041*	25.0933381	1.79578E-10
	Red Brahman	210.4866*	23.1306316	1.79578E-10
	Gyr	97.3168*	24.5543638	0.036253688
	Gyr-Zebu	284.721401	86.222973	0.266558021
	Half-blood Simmental	348.6103*	62.249548	1.75683E-05
	Brown Swiss	212.7908*	54.636595	0.046225644
	Simbrah	175.3517*	39.3409403	0.005136523
White Brahman	Brahman	-230.1658*	32.0544901	9.68916E-10
	Gyr	-132.8490*	31.609878	0.014680834
	Brown Swiss-Zebu	-346.0333*	84.3729205	0.021764871
Grey Brahman	Brahman	-289.0041*	25.0933381	1.79578E-10
	Brahman-Gyr	-280.1261*	56.8004965	0.00058137
	Creole	-288.2430*	69.9316028	0.020122221
	Gyr	-191.6873*	24.5228397	1.86846E-10
	Girolando	-199.3620*	32.8145047	1.10824E-06
	Brown Swiss-Zebu	-404.8716*	81.9815528	0.000562368
Brahman-Gyr	Grey Brahman	280.1261*	56.8004965	0.00058137
	Half-blood Simmental	339.7323*	80.4464232	0.01354427
Red Brahman	Brahman	-210.4866*	23.1306316	1.79578E-10
	Gyr	-113.1698*	22.5104457	0.000362856
	Girolando	-120.84459	31.3391484	0.052820926
	Brown Swiss-Zebu	-326.3541*	81.402245	0.030698205
Creole	Grey Brahman	288.2430*	69.9316028	0.020122221
	Half-blood Simmental	347.849206	90.1984461	0.052737627

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(I) Breed		Difference of means (I-J)	SEM	Sig.
Gyr	Brahman	-97.3168*	24.5543638	0.036253688
	White Brahman	132.8490*	31.609878	0.014680834
	Grey Brahman	191.6873*	24.5228397	1.86846E-10
	Red Brahman	113.1698*	22.5104457	0.000362856
Girolando	Grey Brahman	199.3620*	32.8145047	1.10824E-06
	Red Brahman	120.844586	31.3391484	0.052820926
	Half-blood Simmental	258.9683*	65.74285	0.039530863
Half-blood Holstein	Brahman	-292.2611*	69.9426635	0.016146484
	Brown Swiss-Zebu	-408.1286*	104.801124	0.04628092
Half-blood Simmental	Brahman	-348.6103*	62.249548	1.75683E-05
	Brahman-Gyr	-339.7323*	80.4464232	0.01354427
	Creole	-347.84921	90.1984461	0.052737627
	Gyr	-251.2935*	62.0217726	0.026230064
	Girolando	-258.9683*	65.74285	0.039530863
	Brown Swiss-Zebu	-464.4778*	99.8313858	0.002164968
Brown Swiss	Brahman	-212.7908*	54.636595	0.046225644
Brown Swiss-Zebu	White Brahman	346.0333*	84.3729205	0.021764871
	Grey Brahman	404.8716*	81.9815528	0.000562368
	Red Brahman	326.3541*	81.402245	0.030698205
	Half-blood Holstein	408.1286*	104.801124	0.04628092
	Half-blood Simmental	464.4778*	99.8313858	0.002164968
Simmental-Gyr	Brahman	-175.3517*	39.3409403	0.005136523

Likewise, the breed Gyr had a higher sperm concentration than Red Brahman, White Brahman, and Grey Brahman, but the sperm concentration was lower than that of the breed Brahman. In turn, the breed Girolando had a higher sperm concentration than the breed Half-blood Simmental and Grey Brahman. Conversely, the breed Half-blood Holstein had a lower sperm concentration than the breeds Brahman and Brown Swiss-Zebu.

On the one hand, the breed Half-blood Simmental had a lower sperm concentration than the breeds Brahman, Brahman-Gyr Colombian criollo, Gyr, Girolando, and Brown Swiss Zebu, and the breed Brown Swiss had a lower sperm concentration than Brahman. On the other hand, Brown Swiss-Zebu Simmental had a higher sperm concentration than other breeds, such as White Brahman, Grey Brahman, Red Brahman, Half-blood Holstein, and Half-blood Simmental, and the breed Simmental-Gyr had a lower sperm concentration than Brahman.

Significant (p<0.05) differences were found in semen volume between some breeds (Figure 5), such as Grey Brahman, which had a higher semen volume than Girolando and Brown Swiss. The crossbreed Brown Swiss-Zebu had a higher semen volume than the breeds Brown Swiss and Romo. Conversely, three-fourth Senepol-Romo had a higher semen volume than the breed Romo (Figure 6). Neither breed nor AZ had a significant (p>0.05) effect on progressive motility (Table 3).

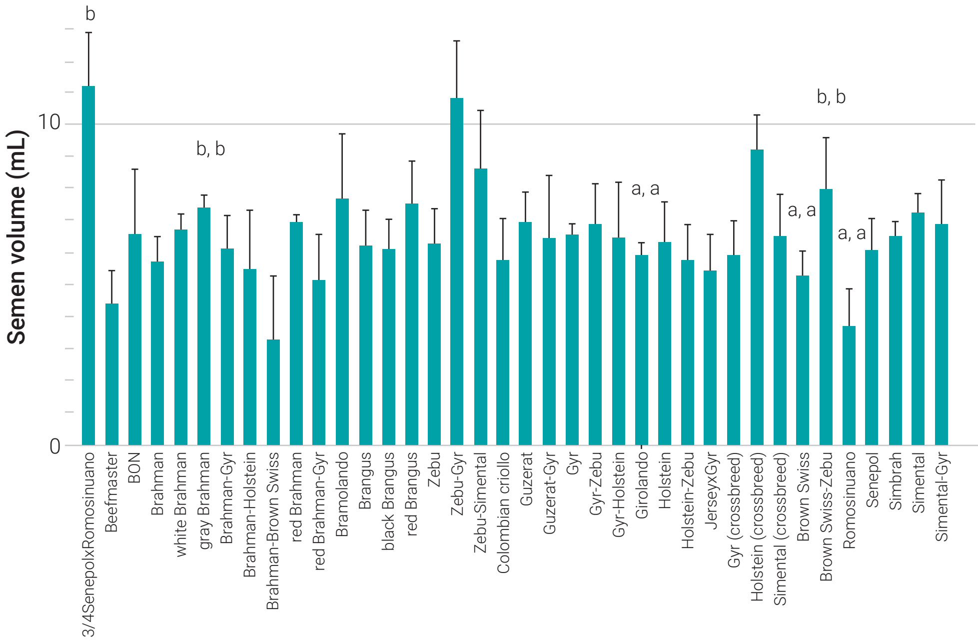

Figure 6. Comparison of semen volumes between breeds and crossbreeds under study

Table 3. AZ and breed effects on progressive motility in bulls of different municipalities of the Cesar department

Origin	Type III sum of squares	gl	Root mean square	F	*p*-value
Adjusted model	54975.213a	117	469.874	1.536	0.000
Intersection	511720.907	1	511720.907	1672.501	0.000
Breed	14367.921	37	388.322	1.269	0.130
AZ	1189.190	4	297.298	0.972	0.422
Breed * AZ	24641.480	76	324.230	1.060	0.344
Error	453434.947	1482	305.962		
Total	7463234.000	1600			
Adjusted total	508410.160	1599			

Note. R squared = 0.108 (adjusted R squared = 0.038)

In turn, in study 2, the following correlations were found: the older the bull, the higher are the scrotal circumference and semen volume (p<0.01) and the higher the semen volume and sperm concentration, the higher is the progressive motility (p<0.01) (Table 4).

Table 4. Correlation between age, scrotal circumference, semen concentration, and volume and progressive motility in bulls of different municipalities of the Cesar department

		Scrotal circumference	Progressive motility	Age	Semen volume	Sperm concentration
Scrotal circumference	Pearson product-moment correlation coefficient	1	-0.013	0.128**	0.026	0.020
	Sig. (bilateral)		0.604	0.000	0.275	0.434
	N	1874	1635	1508	1718	1537
Progressive motility	Pearson product-moment correlation coefficient	-0.013	1	-0.010	0.252**	0.279**
	Sig. (bilateral)	0.604		0.717	0.000	0.000
	N	1635	1710	1376	1687	1566
Age	Pearson product-moment correlation coefficient	0.128**	-0.010	1	0.201**	0.040
	Sig. (bilateral)	0.000	0.717		0.000	0.150
	N	1508	1376	1597	1461	1276
Semen volume	Pearson product-moment correlation coefficient	0.026	0.252**	0.201**	1	0.025
	Sig. (bilateral)	0,275	0.000	0.000		0,321
	N	1718	1687	1461	1796	1572
Sperm concentration	Pearson product-moment correlation coefficient	0.020	0.279**	0.040	0.025	1
	Sig. (bilateral)	0.434	0.000	0.150	0.321	
	N	1537	1566	1276	1572	1590

** The correlation is significant at a 0.01 level (bilateral).

Discussion

Studying the interactions between genotype and environmental conditions is crucial, especially in the tropics, where changing weather conditions resulting from global warming have adversely affected the reproductive performance of animals.

In Colombia and in the tropics, ET and RH depend on the altitude, and some regions with low altitude have a high humidity temperature index [5,6].

Once farm animals reach maturity, sperm production continues throughout their reproductive lives. During sexual rest periods, the old spermatozoa that remain in the epididymis die, degenerate, and are absorbed [7]. Therefore, after a long period of sexual inactivity, semen samples may contain a high percentage of dead and abnormal sperm. By contrast, stated that adult bulls with adequate sexual rest periods and handled calmly usually produce a high volume of good-quality semen by electroejaculation with a high sperm concentration [8].

The rest period in this study did not affect sperm concentration or semen volume, possibly because in the farms, despite the long rest periods, the frequency of periods shorter than 3 days was not high, which would mainly affect these semen characteristics.

The present study evidenced environmental effects on sperm concentration, corroborating other findings reported by Lozano et al. [9], who mentioned that under moderate environmental temperatures (20°C), the testicle is at the limit of testicular hypoxia in which an increase in ambient temperature increases the testicular temperature, metabolic rates, and thus, oxygen requirements. *Bos indicus* bulls experienced thermal stress in the winter, which affected the quality of the semen collected during the season [10]. Nevertheless, productive and/or reproductive problems associated with low temperatures are not commonly found in tropical countries and no technical and/or scientific reports supporting this hypothesis have been published [11].

Assessed seasonal (winter and summer) effects on the semen volume of eight 75% *Bos indicus* x 25% *Bos taurus* breeding bulls, with ages ranging from 20.5 to 27 months, but found no significant differences between summer and winter [12]. Instead, found that the season of the year influenced semen volume or concentration or on sperm motility in Bos Taurus, Bos Indicus, Crossbred and Tropically adapted bulls [13]. However, the percentage of abnormal spermatozoa was significantly higher, and sperm concentration was lower in the warmer months.

No significant difference was found when comparing semen samples of *Bos indicus* and *Bos taurus* bulls [14]. However, in the present study, significant differences were identified in semen volume between taurine and Zebu breeds, indicating that Grey Brahman had a higher semen volume than Girolando and Brown Swiss, in contrast was reported that semen volume was always higher in *Bos taurus* bulls [15].

Given the elevation of large areas in Cesar, this department has various climates, with temperatures ranging from 20 to 30°C and higher, which directly affects the thermoregulatory mechanisms of the testes. In turn, the Institute of Hydrology, Meteorology, and Environmental Studies (IDEAM) et al. [6] reported that the months with the lowest water supply in the department were January, February, and March, directly affecting the availability of forage in the department because the available forage decreases during drought, as reflected in the weight of cattle and their reproduction rates [16].

In this study, significant correlations were found between scrotal circumference and age, showing that the older the bull, the higher the scrotal circumference, in line with the results reported by Cuéllar et al. [17] in other findings were observed the same correlation in crossbred bulls with a coefficient of 0.72 ($p>0.001$). Therefore, scrotal circumference is apparently involved in the secretion of gonadotropins and metabolic hormones because, during the first months of life, the testes develop slowly and a higher luteinizing hormone (LH) secretion is associated with a higher SC and a younger age at puberty [18]. Found that scrotal circumference was positively and strongly correlated with the variables age, progressive motility, and vitality in Guzerat bulls from puberty to 36 months [19].

At the same time, found a strong correlation between age and scrotal circumference in 60 bulls of different breeds and ages, albeit without finding any significant relationship between age and semen volume, in contrast to the present study in which age was significantly correlated with semen [14].

A significant correlation was found between progressive motility and semen volume, whereby the higher progressive motility, the higher is the semen volume; nevertheless, some authors have reported that sperm motility has no effect on semen volume [20].

Seminal volume is made up of sperm and seminal plasma, which is composed of secretions from the accessory sex glands (ampulla, seminal vesicles, prostate, and bulbourethral glands). Most of the secretions in bulls come from seminal vesicles [21]. Among the functions of seminal plasma is that of a nourishment medium for spermatozoa, favoring their transport, and in turn, they play an important role in physiological mechanisms such as sperm-oocyte capacitation and interaction [22]. Plasma is composed of ions, energy substrates (in the case of bulls, fructose is in greater proportion), organic compounds, peptides, and proteins. These proteins present in seminal plasma are crucial for efficient fertilization because they promote the stability of the sperm plasma membrane and motility [21].

Regarding energy substrates, this influences sperm capacitation, with fructose being responsible for generating progressive mobility and glucose for hypermobility [23].

A mechanism that may explain the effect of fructose on progressive motility is that it weakly activates hexokinase [24]; this event allows us to deduce that fructose participates in glycolysis via the fructose phosphate aldolase pathway [25], producing sufficient amounts of ATP to stimulate progressive sperm motility for long periods without causing sperm hyperactivation, similar to lactate and pyruvate [23].

A study found a relationship between testicle size and sperm quality, in addition, the semen volume increased with the testicle size. In this work, the lowest range of semen volume (from 0 to 1.99 ml) included no bulls with a scrotal circumference greater than or equal to 35 cm [8]. Conversely, at the other extreme, bulls with a semen volume ranging from 6 to 8 ml have scrotal circumferences greater than 36 cm. These findings indicate that testicular size is strongly correlated with semen volume, as stated previously [26]. However, the results of the present study did not show a significant correlation of between scrotal circumference and semen volume. Semen volume varies significantly with age since the older the bull, the higher is the semen volume, in line with the results reported by other authors [27]. These authors mentioned that semen volume was positively correlated with age ($r=0.62$; $p<0.01$), increasing approximately 0.5 ml per year, and bulls younger than 1 year produced the lowest semen volumes, whereas bulls older than 4 years produced the highest semen volume.

The semen volume of a young bull was approximately 2 ml, whereas the semen volume of an adult bull ranged from 4 to 12 ml, adding that a bull older than 2 years should not have a semen volume lower than 4 ml [22]. Unsurprisingly, bulls younger than 1 year had the lowest semen volumes [28] because the increase in semen volume with age may be related to the increase in the activity of the hypothalamic-pituitary-testicular axis and simultaneous development of the testes and accessory glands with sexual maturity, which is thought to continue for up to 5 years after puberty [29].

In turn, was identified significant differences in sperm concentration between the breeds *Bos taurus* and *Bos indicus* and crossbreeds [30]. Similarly, in the present study, significant differences in sperm concentration were found between breeds, possibly because the racial percentage of each cross was unknown, the animals had different ages, and the nutritional and environmental conditions likely differed between each bull under study.

In conclusion, the elevation of the altitudinal zones of the Cesar department significantly affects sperm concentration and semen volume. However, neither sperm concentration nor semen volume showed any marked difference by breed, which may have resulted from the heterogeneity of the animals evaluated in this study. The majority of the Bos *Indicus* breeds had a higher sperm concentration than *Bos Taurus* in tropical environmental conditions. Ejaculate with high volume and sperm concentration is observed greater progressive motility.

Conflict of Interest

There were no conflicts of interest that could be perceived as detrimental to the impartiality of the reported research.

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