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Season and Breed Effect in Fresh Semen Parameters at a Bull Semen Production Center in Peru

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ABSTRACT

In the present study, the effect of breed, season, and their interaction on semen volume, sperm concentration, and motility was tested. Nine bulls were tested: three Braunvieh, three Simmental (*B. taurus*), and three Gyr (*B. indicus*). These bulls were managed equally in a cold semi-arid climate, divided into hot (from November to April) and cold (from May to October) for maximum and minimum temperature and relative humidity. Semen was collected twice a week for 24 consecutive months. MANOVA by ranks was used to determine significant effects; breed had a significant effect (P<0.001) on semen parameters, and seasonal variations were not significant (P>0.05). However, there was a significant effect (P<0.001) for the interaction between breed and season. To evaluate differences between breeds, we performed Tukey's post-hoc test; all pairwise comparisons (Braunvieh-Gyr, Braunvieh-Simmental and Gyr-Simmental) showed significant differences (P<0.001). Nonparametric one-way analysis showed significantly higher *B. taurus* breeds' sperm concentration and *B. indicus* breed's semen volume. For interaction between breed and season, Gyr reported higher sperm volume than *B. taurus* breeds in both seasons (P>0.05), but *B. taurus* breeds had higher sperm concentration in the cold season. The findings will help management make better decisions in semen collection, especially during peak season.

Key words: Bulls, Semen, Breed, Season, Effect

INTRODUCTION

Artificial insemination (AI) is a highly used biotechnology for genetic improvement, allowing producers to use superior sires with many females (Murtina et al. 2012; Parkinson and Morrell 2019). AI is designed to facilitate fertilization control and has been the most widespread, least costly and easily accessible to Peruvian farmers. To support the implementation of AI in Peruvian regions, a Genetic Nucleus for semen production was established in Huaral – Lima, Peru (128 masl; 11°31′18″ S and 77°14′06″ W). In Peru, Creole cattle are the most predominant cattle population (64.03%) (Instituto Nacional de Estadística e Informática 2012) and the breeding strategy of small farmers is to crossbreed these animals with specialized breeds such as Simmental, Gyr and others (Rivas et al. 2007), taking advantage of the heterosis effect (Williams and Anderson 2019). That is why the main objective of the production center is to ensure the sufficient quantity and quality of semen supply.

The assessment of fertility post-artificial insemination necessitates a meticulous evaluation of sperm quality (Morrell et al. 2017), involving its ability to migrate, penetrate the ovum, and activate it for embryo formation (Sabés-Alsina et al. 2019). When assessing sperm quality in bulls, critical factors to consider include sperm count, motility, morphology, viability, acrosome integrity, concentration in seminal plasma, DNA integrity, and seminal fluid characteristics (Sankhi et al. 2018; Kumaresan et al. 2020). These parameters collectively serve as indicators of the reproductive capacity of a bull, influencing its potential for successful fertilization. A high sperm concentration, progressive motility, normal morphology, and intact acrosomes indicate good - quality sperm. Additionally, assessing viability, DNA integrity, and seminal fluid characteristics provides comprehensive insights into the overall health and fertility potential of the

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sperm (Allouche et al. 2017; Morrell et al. 2017; Narud et al. 2022). Regular evaluation of these parameters through semen analysis is essential in cattle breeding programs to ensure optimal reproductive performance and successful artificial insemination or natural mating.

Sperm motility has traditionally been used as an indicator of fertility (Foote 2003), but bull sperm characteristics and morphology are influenced by a multifaceted interplay of genetic, environmental, and management factors (Brito et al. 2002; Koivisto et al. 2009; Snoj et al. 2013; Malama et al. 2017). Since spermatogenesis is the intricate process of sperm production that occurs within the seminiferous tubules of the testis, and various factors such as nutrition and environmental conditions can influence the efficiency of spermatogenesis in bulls, this highlights the importance of optimal conditions for maintaining robust reproductive performance in livestock breeding programs (Staub and Johnson 2018; Harrison et al. 2022).

The season can impact semen quality, but there is no consensus between studies. Researchers found out in northern Spain that Holstein bull sperm quality was affected by season, with better values during spring than in winter (Sabés-Alsina et al. 2017), but a study in Brazil with different breeds showed no effect of ambient temperature, humidity or season on sperm production and semen quality (Brito et al. 2002), on the other hand, Simmental bulls in Brazil reported a higher sperm defects during summer than in winter (Nichi et al. 2006). It is essential to know the effect of each of these factors to ensure the success of AI. There has been previous research on the seasonal fluctuations in spermatozoa morphology (Koivisto et al. 2009), ejaculate volume and sperm concentration in the semen of bulls (Wildeus and Hammond 1993) but there is disagreement on the results due to scrotal thermoregulation and heat dissipation mechanisms of bulls (Netherton et al. 2022; Capela et al. 2022). Therefore, the present study aimed to determine seasonal and genotypic effects on sperm motility, sperm concentration and volume by monitoring bulls in an AI center for 24 months.

MATERIALS AND METHODS

Ethical approval

The semen sample collection from the cattle was conducted in accordance with Peruvian National Law No. 30407, "Animal Protection and Welfare."

Season

This work was done in the Central Genetic Nucleus for Bovine, located in the Agricultural Research Station Donoso (EEA Donoso in Spanish), Huaral - Lima, Peru. Data collected from May 2021 to April 2023 were categorized according to two seasons of collection: Hot (November, December, January, February, March, April) and Cold (May, June, July, August, September, and October). This division in the season was made considering the mean temperature and relative humidity recorded by the National Service of Meteorology and Hydrology of Peru (SENAMHI in Spanish); data was collected from the closest weather station to the Central Genetic Nucleus.

Animal management and semen collection

Bulls (from 4 to 6 years old) of the breeds Braunvieh (BU), Gyr (GI) and Simmental (SM) were kept under uniform feeding and management conditions during each season. Bulls were housed in indoor stalls, fed twice daily, and had *ad libitum* access to water and feed. Semen samples were collected from these nine bulls (three BU, three GI, and three SM) through an artificial vagina twice a week, and the temperature of the artificial vagina was varied for each bull (ranging from 56 to 62°C). All bulls were sexually stimulated using a teaser bull or cow, and semen collection was performed by well-trained technicians using a standard protocol with a standard checklist. The method of stimulation and collection was similar for all collections and remained constant from year to year.

Assessment of sperm parameters

The ejaculate samples from each bull were carefully labeled, and the sperm concentration was measured using a photometer (Minitube®). Furthermore, the volume was measured with a micropipette of 1000mL and each sample was pre-diluted with commercial diluent OptiXcell ((IMV Technologies, France), according to the concentration in 1:1 (< 500 sperm/mL) or 1:2 (501 - 1200) or 1:3 (>1300). The pre-diluted samples were then transported to the laboratory (15 minutes from the farm) at 28°C, and the individual motility of the spermatozoa was measured by phase-contrast microscopy and expressed as the proportion of motile spermatozoa (percentage). Immediately, the dilution is completed to obtain the final concentration (20x106spz/0.25mL) to continue the cryopreservation process of the semen.

Semen cryopreservation

Samples with sperm motility that were more outstanding than 60% were considered for the cryopreservation process. Any ejaculation that did not meet this criterion was excluded from the production of genetic material, but the data were still included in this study. After final dilution, samples were incubated at 5°C for five hours. Then, each sample per bull and consecutive ejaculate number was routinely filled and sealed into straws (0.25cc) using an automatic semen filling and sealing machine (Minitube). The straws were placed in a programmable freezer (from 5 to -100°C), and then the straws were rapidly immersed in liquid nitrogen. Three straws from each ejaculate of each bull were thawed at 37°C for 45s in a water bath. The thawed samples were immediately evaluated by standard microscopy, and only those with motility more outstanding than 35% were stored.

Statistical analysis

All semen variables (semen volume, sperm concentration, and motility) and factors (breed, season, and breed-season interaction) were analyzed with R software version 4.3 (R Core Team 2021), using the Rank MANOVA method of the rank MANOVA package (Dobler et al. 2017), and multiple means were compared using the Tukey test of the same package. Also, a linear discriminant analysis (LDA) was done with the ggplot package (Wickhan 2016) to have a graphic representation of the results. Also, we used a nonparametric one-way analysis of variance to address potential differences between the significant effects found in the first analysis, and posthoc analysis was performed using Dwass-Steel-Chritchlow-Figner pairwise comparisons; these last analysis was done with the JVM R package (Selker et al. 2023).

RESULTS

Season

From May 2021 to April 2023, the closest weather station to the EEA Donoso, reported the mean temperature (Fig. 1) and humidity (Fig. 2). Mean temperature varies from 13.2-22°C in cold season and from 14.8-27°C in hot season and humidity varies from 71.7-98% in cold season and from 80.2-98.3% in hot season.

Sperm parameters

Table 1 summarizes ejaculate characteristics of Braunvieh (BU), Gyr (GI) and Simmental (SM) for each season. The mean ejaculate volume ranged from 3.6 to 7.26mL and 3.79 to 6.24mL in cold and hot seasons, respectively. Mean sperm concentration varied between 967.18 to 1304.97 x 10^6/mL and 1109.3 to 1181.4 x 10^6/mL in cold and hot seasons, respectively. Mean sperm motility varied from 54.2 to 60.3% and 57.5 to 61.1% in cold and hot seasons.

Table 1: Summary of the ejaculate characteristics of the three breeds for each season.

Season	Breed	Sperm concentration (10^6 /mL)	Semen volume (mL)	Sperm motility (%)
Cold	BU	1304.97±335.5	3.6±1.3	58.6±11.2
	GI	967.18±366.24	7.26±3.7	54.2±11.9
	SM	1181.4±375.6	4.59±1.7	60.3±12.8
Hot	BU	1175.16±380.6	3.79±1.6	57.5±11.9
	GI	1109.3±341.0	6.24±2.6	61.1±10.0
	SM	1208.4±338.9	4.42±2	60.4±10.4

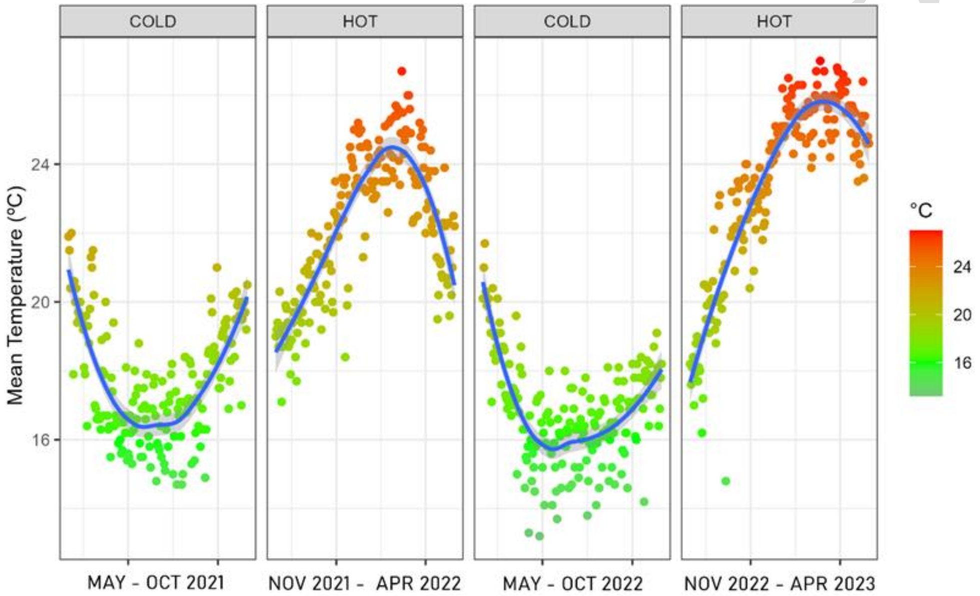

Fig. 1: Mean temperature from May 2021 to April 2023, data were separated into hot and cold seasons.

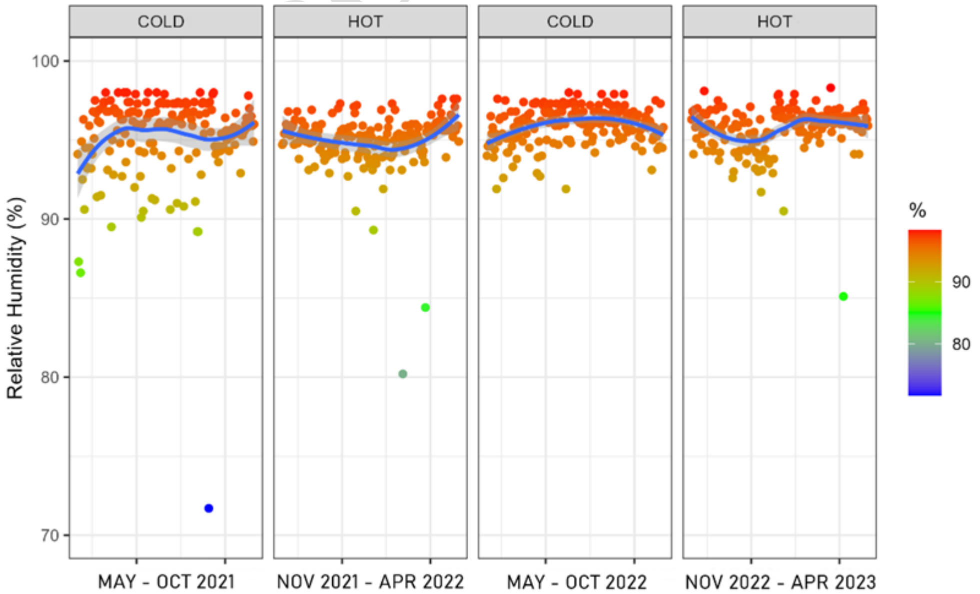

Fig. 2: As described before, data on relative humidity from May 2021 to April 2023 were separated into hot and cold seasons.

Statistical analysis

Multivariate analysis of variance

This study evaluated the effect of breed, seasonal variations and their interaction (Table 2) in semen quality in BU, GI and SM, where the null hypothesis (H_0) means that all mean vectors are equal and the alternative hypothesis means that at least one mean vector is different from the rest. Breed had a significant (P<0.001) effect on the semen parameters. By obtaining a P<0.001, the null hypothesis (H_0) is rejected, meaning there are significant differences between the three breeds. Therefore, it is assumed that at least one vector of means is different from the rest. Also, a significant (P<0.001) effect exists on the interaction between breed and season. However, seasonal variations are not significant (P>0.05), and the null hypothesis (H_0) is not rejected, which means that there are no significant differences between the two seasons studied.

Table 2: Effect of breed, seasonal variations and their interaction.

	Statistic test	P value
Breed	81.219	P<0.001***
Season	10.267	0.054
Breed:Season	150.531	P<0.001***

***highly significant (P<0.001).

Tukey post-hoc analysis

The effect of breed, seasonal variations and their interaction were evaluated, as the breed effect was significant; since we do not know which treatment this different mean corresponds or which treatments are different from each other, we proceed to perform the Tukey post-hoc test (Table 3), all pairwise comparisons showed significant differences (P<0.001).

Table 3: Pairwise comparisons between breeds.

	Statistic test	P value
GI – BU	62.998	P<0.001***
SM – BU	72.373	P<0.001***
SM – GI	85.785	P<0.001***

***highly significant (P<0.001).

Linear discriminant analysis (LDA)

LDA was performed to maximize the separation between groups. The effect of the breed (Fig. 3) shows that the groups are not very far apart. However, the rankMANOVA method is usually susceptible to small changes (Dobler et al. 2020), so this slight separation is already detected as a significant difference.

Although the season was not significant, we performed the LDA (Fig. 4) to support our results. In this case, the groups are much closer to each other than in the previous case, which is why the rank MANOVA did not detect significant differences.

Nonparametric one-way analysis of variance

Nonparametric one-way analysis of variance revealed significant differences between breeds for sperm concentration (P=0.0001809) and semen volume (P<0.0000001) but no significant differences were determined for motility (P=0.0773065) (Table 4).

Dwass-Steel-Chritchlow-Figner pairwise comparisons post-hoc analysis

Post hoc analysis with Dwass-Steel-Chritchlow-Figner pairwise comparisons between breeds showed

Table 4: Differences between breeds for each semen characteristics

	χ^2	df	P Value
Sperm concentration	17.23485	2	P<0.0001809***
Semen volume	109.1618	2	P<0.0000001***
Sperm motility	5.119954	2	P<0.0773065

***highly significant (P<0.001).

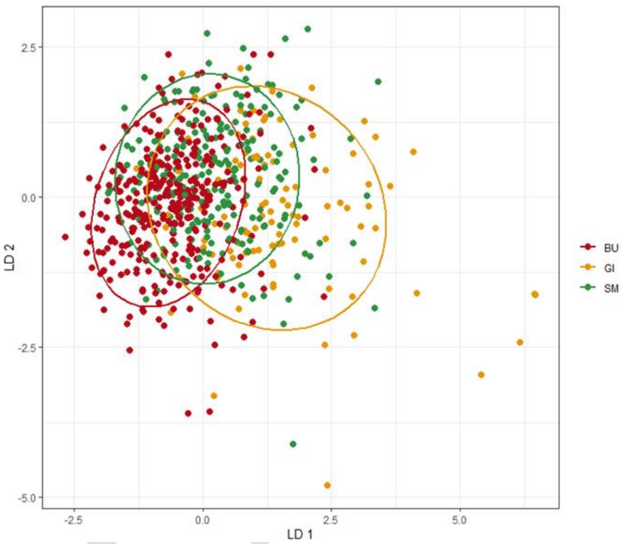

Fig. 3: Linear discriminant analysis of breed's effect.

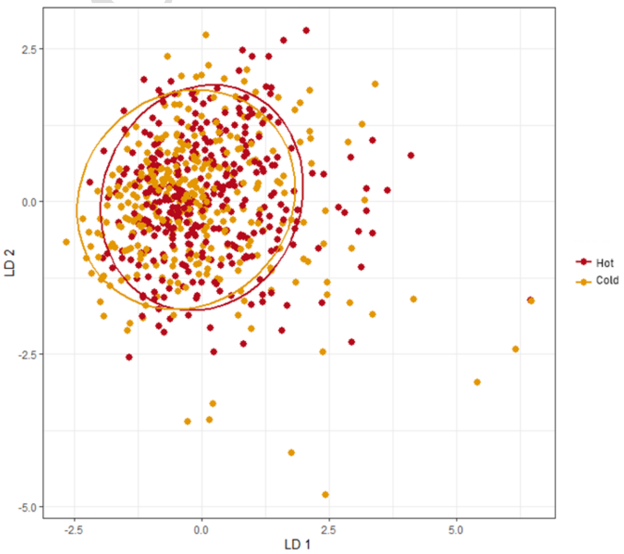

Fig. 4: Linear discriminant analysis of season's effect.

significant differences in BU-GI (P=0.0005795) and GI-SM (P=0.0001929) for sperm concentration and BU-GI (P<0.0000001), BU-SM (P=0.0000075) and GI-SM (P<0.0000001) for semen volume, however, there was no evidence of a significant difference in sperm concentration between BU-SM (P=0.9763773) (Table 5).

Pairwise comparisons between the interaction of breeds and season, showed significant differences in all of the pairwise in cold season for semen volume and only in BU-GI and GI-SM pairwise for sperm motility, however there was no evidence of a significant difference in hot season for any pairwise for sperm concentration and motility (Table 6).

Table 5: Pairwise comparisons between breeds

	Sperm concentration		Semen volume		Sperm motility	
	W	P value	W	P value	W	P value
BU - GI	-5.26426	P<0.0005795***	14.120908	P<0.0000001***	1.192111	P<0.6765349
BU - SM	-1.92821	P<0.9763773	6.658369	P<0.0000075***	3.187561	P<0.0624943
GI - SM	5.21325	P<0.0001929***	-10.22086	P<0.0000001***	1.252697	P<0.6493779

***highly significant (P<0.001).

Table 6: Pairwise comparisons between the interaction of breeds and season

		Sperm concentration		Semen volume		Sperm motility	
		W	P value	W	P value	W	P value
Cold	BU - GI	-5.4374	P<0.001686**	9.068784	P<0.0000001***	-2.81251	P<0.348697
	BU - SM	-1.32915	P<0.936316	4.875442	P<0.0074886**	1.400486	P<0.921435
	GI - SM	4.97026	P<0.005893**	-7.01666	P<0.0000104***	4.27442	P<0.098119
Hot	BU - GI	-2.26967	P<0.595356	10.51378	P<0.0000001***	3.672223	P<0.174508
	BU - SM	1.42599	P<0.915481	4.61322	P<0.0141200*	2.80376	P<0.352406
	GI - SM	3.49019	P<0.133827	-7.39775	P<0.0000025***	-0.40953	P<0.9997279

*significant (P<0.05), ** significant (P<0.01), ***highly significant (P<0.001).

DISCUSSION

Consider the present work as the first report that evaluates the effect of breed, seasonal variation and their interaction on seminal volume, sperm concentration and motility in the Central Genetic Nucleus - EEA Donoso. In Peru, artificial insemination was introduced in the 1940s and today it is highly applied in different parts of the country, especially in dairy cattle, used strategically to accelerate genetic improvement and to maximize reproductive efficiency, breeders must be aware of the limits and capabilities of a bull's reproductive ability. The quality of semen in bulls can be influenced by both breed characteristics and seasonal variations, as well as the interaction between them (Brito et al. 2002; Koivisto et al. 2009; Sabés-Alsina et al. 2017). Different breeds exhibit varying levels of fertility and semen quality, with genetic factors playing a significant role (Wildeus and Hammond 1993; Mathevon et al. 1998; de Lucio et al. 2014). In this study, the breed had a significant effect in sperm concentration and ejaculate volume, Braunvieh (BU), Gyr (GI) and Simmental (SM) individuals were tested.

There were significant differences between SM and BU compared to GI in sperm concentration and ejaculate volume, this may be because SM and BU are *B. taurus* breeds while GI is a *B. indicus* breed, also, non-significant difference in sperm concentration may be explained by the same reason. A previous study had reported higher sperm concentration in *B. indicus* bulls than in *B. taurus* bulls but smaller ejaculate volumes (Brito et al. 2002), on the contrary, our results pointed that BU and SM sperm concentrations were higher than GI, but GI had higher ejaculate volume than BU and SM. The previous study was done in Brazil, where *B. indicus* bulls show a higher performance because of their better thermoregulatory capabilities, also it was done with a majority of other breeds (Holstein, Red Angus, Aberdeen Angus, Limousin and Nelore), while it only had one GI and two SM included, this could explain the differences found in our study.

Sperm motility was not significantly affected by breeds, similar to Wildeus and Hammond (1993), Rekwot et al. (1987) and Brito et al. (2002) studies which reported that no significant differences were found between *B. taurus* breeds and for Zebu cattle, but in other study (Wildeus and Hammond 1993) higher motility was determined in *B. indicus*, possibly due to the tolerance to heat stress attributed to *B. indicus* breeds (Riley et al. 2012), which may be because our study was on a temperate climate. For the Simmental breed, Novianti et al. (2020) reported that compared to Indonesian indigenous breeds, Simmental had lower sperm motility with 65.959%±4.45%, which is higher than the mean sperm motility value in the present study. To sum up, BU and SM had significantly higher sperm concentration than GI on pairwise comparison, while semen volume was the opposite, GI had significantly higher values than BU and SM. Conversely, sperm motility differences were not significant for any pairwise comparison.

Seasonal fluctuations, can include changes in temperature, photoperiod, and nutritional status, can impact reproductive hormone levels and semen quality, additionally, heat stress, common in hotter months, can further reduce semen quality (Capela et al. 2022; Netherton et al. 2022). In our study, only temperature was considered and two seasons (hot and cold) were evaluated. The effect of seasonal variation was not significant, but if we look at the P-value (0.054), it is very close to 0.05, which is the cut-off point, meaning it is very close to being significant. Despite this result, there is no consensus about the seasonal effect in bull sperm characteristics; some studies show seasonal effects (Söderquist et al. 1996; Vilakazi and Webb 2004; Nichi et al. 2006; Murphy et al. 2018; Nongbua et al. 2020) and others do not (Mathevon et al. 1998; Brito et al. 2002). Other factors, such as local adaptation of cattle, individual thermoregulation, and genotype x environment interactions, may be attributed to these differences. Brito et al. (2002) in a study conducted in Brazil, found that there was no significant effect of ambient temperature or humidity on sperm production and semen quality; they mentioned that this is consistent with another study (Everett and Bean 1982) conducted in temperate environments, both studies have temperatures and humidity similar to ours. In addition, the seasonal effects could be attributed to temperature and humidity, the length of the day and management (Fuerst-Waltl et al. 2006), variations are expressed differently in different locations according to latitude. It would also be interesting to evaluate the spermatozoa's morphology, which has been reported to have an effect at higher temperatures (Seifi-

Jamadi et al. 2020). Our results might be because bulls take around 60 days to complete spermatogenesis (Staub and Johnson 2018), therefore, changes in ejaculate characteristics brought on by unfavorable circumstances, like variations in testicular temperature, would not be immediately noticed. Therefore, it depends on the time the assessment of sperm quality is done, and the impact of climate on sperm quality could go unnoticed.

Our study's effect on the interaction between breed and season was significant; previous work has obtained similar results (Chacón et al 2002; Teixeira et al. 2011; Landaeta-Hernández et al. 2020; Llamas-Luceño et al. 2020), but studies had a different methodology, some of them use a temperature-humidity index (Llamas-Luceño et al. 2020) or monthly temperatures (Teixeira et al. 2011) while others use month-established seasons (Landaeta-Hernández et al. 2020), in our case, temperature-humidity index did not seem to be ideal because our humidity levels do not fluctuate as in other regions. Our findings showed lower sperm motility of *Bos Taurus* bulls (BU and SM) during the hot season; this could be due to lower heat tolerance of these animals in contrast to the innate tolerance of Gyr, however our results did not find these differences to be significant. As mentioned above, the seasonal effects could not only be attributed to temperature and humidity; high temperatures and poor pasture quality have been reported to affect sperm volume (Koivisto et al. 2009), unlike our study, where all bulls were adequately fed with the same diet. In cold season, BU and SM presented significantly higher sperm concentration than Gyr, this could be because Gyr is a tropical cattle, on the contrary, Gyr had significantly higher semen volume than the *Bos taurus* breeds, Koivisto et al. (2009) also reported significantly higher values for *Bos indicus* breeds in all four seasons of their study. On the other hand, in hot season, only Gyr reported significantly higher semen volume than BU and SM, the reason for this might be that these breeds don't tolerate heat as well as Gyr does (Hansen 2004). In a study (Freitas et al. 2020), there was a significant effect of the temperature-humidity index in Gyr, high temperatures had negative effects on semen characteristics but they mentioned that the minimum temperature during the night balanced these negative effects, this could explain that Gyr values for sperm volume were higher than the other breeds in both seasons, plus zebu bulls' ability to control heat exchange and sustain spermatogenesis (Chacón et al. 2002).

Although breed, season and their interaction's effect were analyzed, it has been observed that the timing of sexual preparation significantly influences the volume, number of doses per ejaculate, and post-thaw motility of ejaculates, in AI centers, semen collection crew are in charge of sexual arousal and preparation, this could help to explain why bull handlers and semen collectors have a significant influence on the quantity and quality of semen (Fuerst-Waltl et al. 2006). A study has shown that the ejaculator equipment and the semen collection crew have a substantial impact on the ejaculate volume but not the sperm concentration and motility (Mathevon et al. 1998), in the present study, semen was collected with the same equipment, but due to the number of years of data collected, it was not performed by the same technician or semen collection crew.

Conclusion

In conclusion, sperm concentration, semen volume and sperm motility of Braunvieh, Gyr and Simmental bulls were studied, results showed a significant effect of breed and breed x season interaction on sperm concentration and semen volume. These results will help to improve the technical aspects of management decisions at Central Genetic Nucleus. Our results underline the necessity of conducting regular evaluations of all the bulls, to increase seminal characteristics data. Further studies are needed to investigate other factors affecting semen characteristics, add pre- and post-freezing data, and provide fertility data.

Author contributions

Conceptualization, D. D-B., S.L. and C.Q.; methodology, D. D-B. and S.L.; data collection (laboratory), D. D-B. and S.L.; data collection (field), R.M. and J.R.; animal management, R.M. and J.R.; software, D.F.; General revision of the manuscript, W.Y.A-G.; Review and editing in English, W.Y.A-G. and D.F.; formal analysis, R.E. and D.F.; writing—original draft preparation, D.F. and D. D-B.; supervision, W.Y.A-G. and C.Q.; project administration, C.Q.; funding acquisition, C.Q. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflicts of interest.

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